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21 JUN 1948

CANADIAN JOURNAL OF RESEARCH

VOLUME 25
1947
SECTION D

CANADA

Published by the
NATIONAL
RESEARCH COUNCIL
of CANADA

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 25, SEC. D. FEBRUARY, 1947 NUMBER 1

STUDIES ON THE LIFE HISTORY OF *TRICHOCEPHALUS VULPIS*, THE WHIPWORM OF DOGS[1]

BY M. J. MILLER[2]

Abstract

Under experimental conditions fully embryonated eggs of *Trichocephalus vulpis* hatch in the small intestine of dogs within 30 min. after the eggs are fed. The larvae penetrate the intestinal mucosa within 24 hr. after infection and at that time may be found deeply embedded. Between 48 and 96 hr. after infection the larvae become coiled and sluggish, and the majority are found lying at the base of the crypts of Lieberkuhn in the upper levels of the small intestine. They do not work their way back to the lumen of the intestine for at least eight days, although there is some evidence that they may return to the lumen by the 10th day after infection and are then carried passively down to the large intestine. There is some minor tissue destruction by the larvae during their mucosal penetration.

Introduction

Davaine (2) did the first experimental work on the life history of whipworms. He showed that eggs of *Trichocephalus trichiurus* were passed in an undeveloped state and required a period of eight and a half months to embryonate. Leuckart (6) was able to embryonate eggs in four to five months and proved that the life cycle of the whipworm did not require an intermediate host. He fed embryonated eggs of *T. ovis* to a lamb; 16 days later he sacrificed the animal and at autopsy found numerous larvae in the caecum. He repeated the experiment with *T. crenatus* in swine, and was able to recover whipworms, almost mature, four weeks after the eggs were fed. Railliet (10) fed eggs of *T. vulpis* to two dogs; one received embryonated and the other unembryonated eggs. The dog fed embryonated eggs was sacrificed three months later and numerous whipworms were found, some of the females passing eggs. The control animal did not become infected.

Following the work of Stewart (11) on the lung migration of the larvae of ascaris, interest was revived in the life history of whipworms. Neshi (9) working with *T. vulpis* in dogs concluded, after feeding embryonated eggs and finding several larvae in the lung tissue, that whipworms required a larval lung migration in their development. Tochihara (12) working with eggs of *T. vulpis* and using dogs and rats as hosts reported that the eggs hatched in the small bowel one hour after ingestion, and that

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[1] *Manuscript received June 12, 1946.*

Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada.

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the larvae remained in the intestine until they were completely developed. He found no evidence of a lung migration of the larvae and stated that development to maturity required approximately one month. Fülleborn (3) working with *T. leporis* in rabbits and a species of whipworms in monkeys could find no evidence of a lung migration of the larvae.

Hasegawa (4) fed embryonated eggs of *T. vulpis* to dogs and followed the larval development. He found that the eggs hatched in the lumen of the small intestine and penetrated the villi of the small bowel where they remained for a period of approximately two days. They never penetrated deeper than the submucosa but remained coiled in the crypts of Lieberkühn, the cells of which were destroyed. Seventy hours after infection, larvae appeared in the caecum, where the number gradually increased; without further migration they developed into adults.

Hirasawa (5) working with *T. vulpis* and *T. trichiurus* could find no evidence of a lung migration of the larvae.

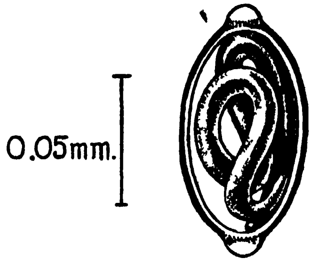
Matsubayashi (7) working with *T. vulpis* in rats, found that eggs hatched in the small intestine one-half hour after ingestion. The larvae then penetrated the mucosa of the small bowel to leave and re-enter at intervals. Finally after a period of from six to nine days, larvae started to disappear from the mucosa and became more numerous in the lumen, shortly after which they disappeared altogether; apparently further development in the rat was impossible.

The present investigation was undertaken in an attempt to elucidate the early course of *Trichocephalus vulpis* infections in dogs, particularly to ascertain whether there is a migration of the larvae through the lungs in the course of their development. The results obtained, while in essential agreement with the majority of references cited above, bring out several interesting points at variance with previous work.

Materials and Methods

Eggs of *Trichocephalus vulpis* used in the experimental infections were collected by the brine centrifugal-flotation method and incubated at 33° to 36° C. in Petri dishes having a diameter of 10 cm. Bacterial growth was kept down by adding a few drops of 10% formalin to the suspension of eggs in water. When the eggs contained well-developed vermiform embryos (Fig. 1), they were removed from the incubator and stored in stoppered bottles at room temperature. The eggs were utilized shortly after reaching their full development and were never allowed to remain in storage for any length of time, as they lost their viability very rapidly. Before eggs were used in any experiment they were tested for viability by crushing them under a cover glass and observing the larvae for motility. Often a batch of eggs was found non-viable and was discarded.

The dogs used in the present investigation were sacrificed either by injecting ether into the heart or by electrocution. Immediately after death the peritoneal cavity was opened and the digestive tract removed. The tract was separated into several sections representing the stómach, duodenum, jejunum, ileum, caecum, colon, and rectum. Each segment was then opened



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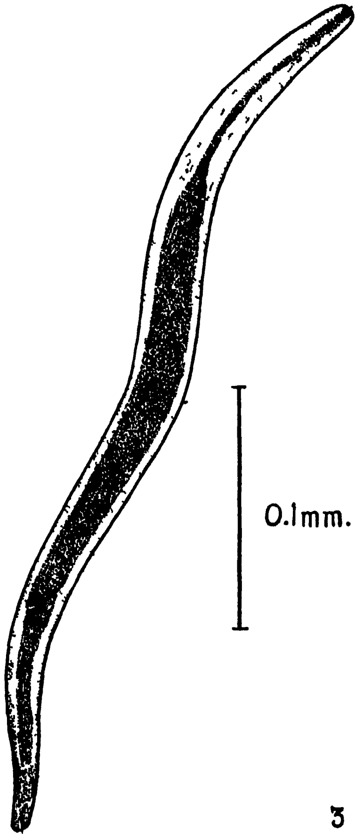

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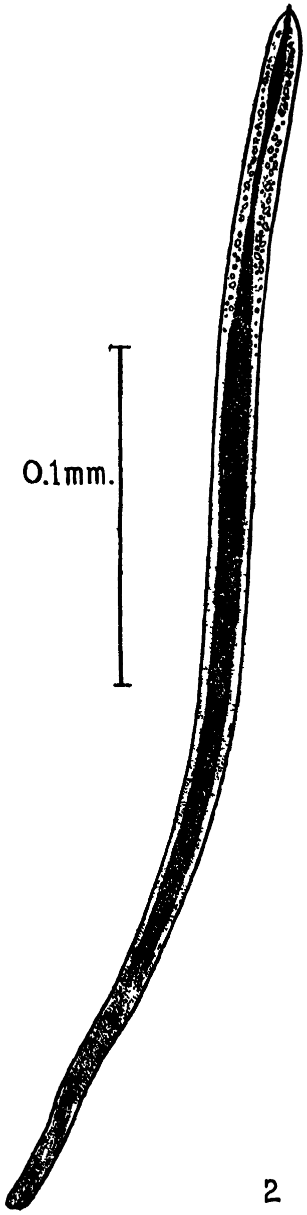

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FIG. 1. *Fully embryonated infective egg of T. vulpis.*

FIG. 2. *Twenty-four-hour-old larva of T. vulpis from superficial mucosal scrapings of duodenum.*

FIG. 3. *Six-day-old larva of T. vulpis from deep mucosal scrapings of jejunum.*

and examined, except that, in animals sacrificed more than 24 hr. after inoculation, the stomach was not included in the examination. Examinations for larvae were conducted in the following manner: first, superficial scrapings of the mucosa were made and the material placed on a large glass slide, thoroughly mixed with physiological saline, another glass slide superimposed, and the preparation carefully searched under 100× magnification. Following this, deeper scrapings of the mucosal wall were made and examined in a similar manner. Usually about a fourth of the entire mucosa was examined in this way. When larvae were found they were studied both alive and after being killed by gentle heating. It was found necessary to use the oil immersion lens in order to study the internal anatomy of the very young larvae. The liver and lungs were examined for larvae by mincing the tissue in physiological saline and examining the sediment of the resulting fluid; in addition, bits of the tissue were compressed between glass slides and examined directly.

During the first 24 hr. of infection five animals were examined, the first, 30 min.; the second, one hour; the third, five hours; the fourth, 10 hr.; and the fifth, 24 hr. after the feeding of eggs. The development of the larvae for the next three days was studied by sacrificing and examining animals after 48 hr., 72 hr., and 96 hr., respectively. After the fourth day, infections were studied every 48 hr. Development was followed till the 10th day after inoculation.

Results

A total of 11 dogs was used in this experiment; detailed information is presented in the following protocols.

Protocol No. 1

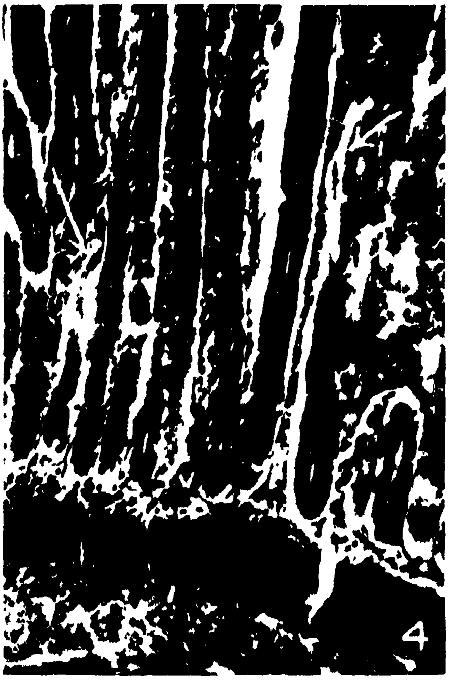
This animal was inoculated with 250,000 fully embryonated, viable *T. vulpis* eggs, and was sacrificed 30 min. after inoculation. *Post-mortem* examination revealed a few embryonated eggs in the stomach, but no larvae. The duodenum contained numerous embryonated eggs, a few eggs in the act of hatching, and a considerable number of larvae. The jejunum and upper part of the ileum contained a fair number of larvae, as well as embryonated eggs. The lower part of the ileum contained a number of embryonated eggs and empty egg shells. The large bowel was negative for eggs or larvae.

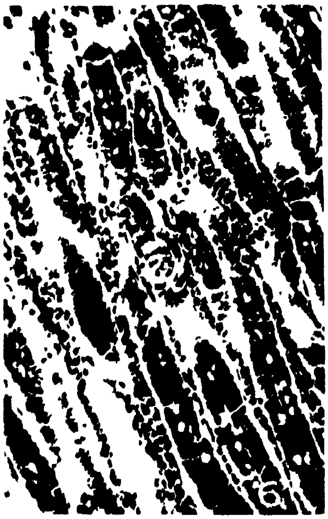
Protocol No. 2

This animal was given 40,000 infective-stage *Trichocephalus* eggs and was sacrificed one hour later. *Post-mortem* examination of the gut revealed numerous unhatched eggs in the stomach. The duodenum contained a few unhatched eggs. The jejunum and upper ileum contained motile larvae and empty egg shells. The lower ileum and upper part of the large bowel contained only embryonated eggs.

Protocol No. 3

This animal was inoculated with 80,000 fully embryonated eggs and was sacrificed five hours later. Examination of the gut showed the stomach to





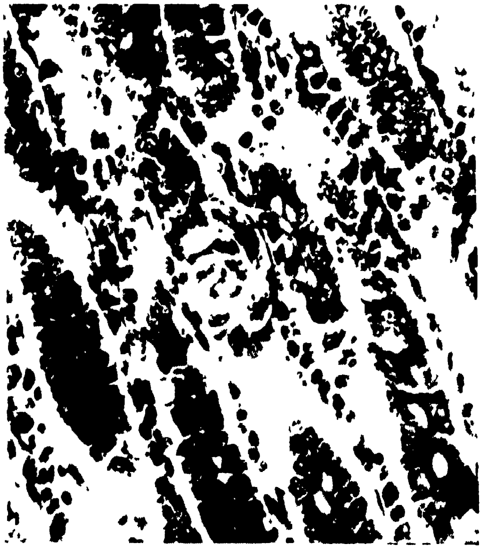


FIG. 4. *Section through middle third of jejunum of dog showing 48-hr.-old T. vulpis larvae in situ. Print overdeveloped to bring out the two larvae indicated by arrows (×95).*

FIG. 5. *Material as above under higher magnification (×195). Note mouth spear at head end of larva, and absence of larval coiling at this stage.*

FIGS. 6 and 7. *Section through upper third of jejunum of dog, showing 96-hr.-old T. vulpis larvae in situ. Note absence of tissue reaction and very slight evidence of tissue damage. At this stage the larva has assumed a coiled position (magnified 95× and 195×, respectively).*

be entirely free of eggs or larvae. A search of the duodenum revealed numerous active larvae, but no unhatched eggs or empty egg shells. The jejunum contained numerous larvae and was free of unhatched eggs or egg shells. A search of the ileum revealed active larvae in small numbers. The distal tip of the ileum was filled with bulky faeces, containing numerous egg shells and a fair number of torpid, very sluggish larvae. The caecum contained egg shells, dead larvae and a few sluggish, living larvae; only one actively motile larva was seen. The remainder of the large bowel contained numerous egg shells and dead larvae.

Protocol No. 4

This animal was inoculated with 300,000 infective-stage eggs and was sacrificed 10 hr. later. *Post-mortem* examination of the gut showed the stomach, duodenum, and upper two-thirds of the jejunum to be free of larvae or eggs. The lower third of the jejunum contained numerous, actively motile larvae. The upper half of the ileum contained a few active larvae, and the lower half a very few active larvae. The caecum contained a few dead larvae and egg shells, but no living larvae. The remainder of the large bowel contained egg shells and unhatched eggs.

Protocol No. 5

This animal received an inoculation of 250,000 infective-stage eggs. Twenty-four hours later it was sacrificed. *Post-mortem* search showed the stomach to be entirely negative. Superficial scrapings of the duodenal mucosa revealed numerous active larvae, while deeper scrapings showed a few active larvae. Larvae were quite numerous throughout the jejunum both superficially as well as in the depths of the mucosa. Superficial scrapings of the upper third of the ileum revealed a small number of larvae and deeper scrapings a larger number. The middle third of the ileum revealed a very few larvae in the superficial scrapings, but none in the deeper mucosa. No larvae were found in the lower third of the ileum or in the large bowel.

The 24 hr. larva had an elongated slender body, and exhibited active motility. It measured about 0.36 mm. in length and 12.5μ in width at its widest diameter. The body was widest at the anterior end. It tapered slightly at the anterior tip and more markedly towards the posterior end of the body. The tail ended bluntly. The alimentary canal consisted of a thin anterior portion, which ran one-fifth of the body length and then gradually broadened into a wider region, which ran throughout the body to end in a well-defined anus at the caudal extremity. The posterior portion of the alimentary tract showed very little differentiation from the surrounding tissues. There was a well defined mouth spear, but no evidence of a genital primordium (Fig. 2).

Protocol No. 6

This animal was sacrificed 48 hr. after inoculation with 250,000 infective-stage eggs. *Post-mortem* examination of the duodenum revealed a few active larvae in the superficial mucosa, and a slightly larger number in the deeper areas of the mucosa. The jejunum revealed numerous active larvae both in the superficial and deeper areas of the mucosa. The upper two-thirds of the ileum showed a reduced number of active larvae in the superficial and deeper scrapings. The lower third of the ileum, the caecum, and the upper reaches of the colon revealed a small number of active larvae in the deeper regions of the mucosa. The lower half of the large bowel was negative. The liver and lungs were negative for whipworm larvae.

The 48 hr. larva resembled the 24 hr. one very closely. It was slightly larger, measuring up to 0.39 mm. in length, and still exhibited considerable motility.

Sections were made of tissue taken from the middle of the jejunum. They revealed larvae in a linear position, situated within the crypts of Lieberkühn, the majority lying between the base of the goblet cells and the basement membrane. There was no evidence of host tissue reaction or cellular destruction at this stage (Figs. 4 and 5).

Protocol No. 7

This animal was inoculated with 250,000 infective-stage eggs and was sacrificed 72 hr. later. *Post-mortem* examination indicated that the duodenum was negative. The upper half of the jejunum revealed one dead, partially encapsulated larva in the lumen mucus, a few dead larvae in superficial scrapings, and a small number of active larvae in the deeper scrapings, of the mucosa. The lower fourth of the ileum revealed a few dead and a few active larvae, both in the superficial and deeper regions of the mucosa. The remaining areas of the alimentary tract were negative. The liver and lungs were negative for whipworm larvae.

The larvae of the 72 hr. infection were very similar to the one- and two-day larvae described above.

Protocol No. 8

Ninety-six hours after this animal received an inoculation of 250,000 infective-stage whipworm eggs, it was sacrificed and examined. The duodenum showed a few active larvae in the superficial scrapings, and a fairly large number in the deeper areas of the mucosa. A search of the upper half of the jejunum revealed a few larvae in the lumen mucus, and a large number in the scrapings of the mucosal tissue. The lower half of the jejunum contained a few larvae in the superficial scrapings, but none were found in the deeper scrapings of the mucosa. The upper third of the ileum was negative, while the lower two-thirds revealed a very few larvae from extensive scrapings of the mucosal layer. Many scrapings of the caecal mucosa revealed one larva. The colon was negative. No larvae were recovered from the liver and lungs.

The four-day larva did not show any marked anatomical changes from the preceding three stages described, although there was a slight thickening of the body. Its motility, however, was greatly decreased and was of a sluggish type. In most cases it had become coiled.

Tissue from the upper third of the jejunum was sectioned. Larvae were found coiled up in the crypts of Lieberkühn. There was no evidence of host tissue reaction, but minor gland tissue destruction could be seen in the immediate vicinity of the parasite (Figs. 6 and 7).

Protocol No. 9

This animal was sacrificed six days after receiving an inoculation of 300,000 infective-stage eggs. *Post-mortem* examination of the duodenum revealed a small number of larvae in the superficial and deeper levels of the mucosa. The upper half of the jejunum contained numerous larvae in the superficial mucosa, and a smaller number in the deeper regions; in the lower half of the jejunum, larvae were numerous both in the superficial and deeper areas; in the upper half of the ileum, there were a few larvae in the superficial scrapings, and a still smaller number in the deeper scrapings of the mucosal tissues; the lower half of the ileum, one larva in superficial scrapings of the mucosa. The intralumen contents of the caecum contained several dead and degenerating larvae. Scrapings of the caecal mucosa revealed a fair number of tightly coiled larvae, showing no motility but living. The colon was filled with faeces, which contained numerous dead and degenerating larvae. Scrapings of the wall of the colon revealed a small number of coiled larvae. The liver and lungs were negative for larvae.

The six-day larva (Fig. 3) showed certain morphological changes from the preceding forms. There was little increase in length, but the body was almost twice as wide as the 24-hr. larva, being about 23μ in diameter. This larva showed the first differentiation of the gut into distinct oesophageal and intestinal regions. The larvae in most cases were in a tightly coiled condition, and exhibited only very sluggish motility.

From this autopsy a portion of the jejunum was sectioned. The sections contained larvae throughout the lower two-thirds of the mucosal layer. The majority occurred in the crypts of Lieberkühn, but not infrequently larvae were seen in the stroma itself. They were never found below the muscularis mucosae. There was no evidence that the larvae provoked any cellular reaction in the host nor was there any sign of tissue encapsulation of the parasite, only evidence of some mechanical damage to the host tissue, and more characteristically, liquefaction of cells in the immediate vicinity of the larvae (Figs. 8 to 14).

The majority of the larvae were lying coiled up. However, occasionally a larva was seen in a linear position in the upper half of the mucosa while in a position directly below it could be seen an area of tissue detritus suggesting that the larva had left its location and was migrating towards the lumen of the bowel (Figs. 13 and 14). This could possibly mean that certain larvae start their migration out of the small bowel mucosa as early as the sixth day following infection.

Protocol No. 10

This animal was inoculated with 300,000 infective-stage eggs, and sacrificed eight days later. An examination of superficial scrapings of the duodenum proved to be negative for larvae. Deeper scrapings, however, revealed numerous larvae showing slightly more motility than the larvae of the six-day infection. Except for the presence of one tightly coiled larva in the superficial scrapings of the lower third of the ileum, the remainder of the gut was entirely negative for larvae. No larvae were found in the liver and lungs.

The eight-day larvae differed little from the six-day forms. However, they appeared to show slightly more motility, and were not as tightly coiled.

A portion of the duodenum taken from this autopsy was sectioned, and revealed numerous larvae concentrated at the base of the crypts of Lieberkühn, and in the adjacent tissues (Figs. 15 to 21). Here, as in the six-day infection, most of the larvae were in a coiled position, but a few larvae were seen in a linear state suggesting a migration towards the lumen of the bowel. The host–parasite reactions were found to be similar to those described above in the six-day infection.

Protocol No. 11

This animal was fed 300,000 infective-stage eggs and sacrificed 10 days later. *Post-mortem* examination revealed one coiled larva in the deep scrapings of the duodenal mucosa, although superficial portions were found negative. Superficial scrapings of the upper half of the jejunum revealed one tightly coiled and four uncoiled larvae. Only one showed slight motility; the remaining four appeared to be dead. The deeper scrapings were negative. Examination of the lower half of the jejunum revealed one coiled larva in the deeper levels of the mucosa. The upper two-thirds of the ileum contained a very small number of apparently dead larvae in the superficial areas of the mucosa. The lumen contents of the lower third of the ileum, however, harboured numerous, mostly uncoiled larvae, exhibiting slight motility. Scrapings of the mucosal wall of this region did not reveal any larvae. Superficial scrapings of the caecum revealed fairly numerous larvae in an uncoiled state, exhibiting slight motility, also a few degenerating forms. In the deeper levels of the mucosa a very few tightly coiled forms were found. A small number of larvae was recovered from the superficial scrapings of the colon. Liver and lungs were negative for larvae.

PLATE II. FIGS. 8 to 14. *Six-day-old larva in situ.*

FIG. 8. *Coiled larvae lying just above the muscularis mucosae. Note disorganization of normal tissue arrangement (×50 and ×175, respectively).*

FIG. 9. *Partially coiled larva (×80). Note relatively minor tissue damage.*

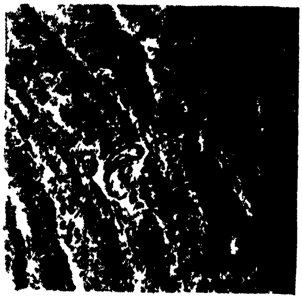
FIG. 10. *Larva lying coiled up in the stroma between the bases of the crypts of Lieberkühn (×175). Evidence of minor tissue destruction.*

FIG. 11. *Enlargement of Fig. 8 (×175).*

FIG. 12. *Larva lying lengthwise in a crypt of Lieberkühn. Note almost complete destruction of gland cells at base of crypt (×175).*

FIGS. 13 and 14. *This larva appears to be migrating towards the lumen of the bowel. Note the disorganization of cells at the base of the crypt represented by dark-staining cellular detritus; this is probably due to the previous localization of the larva (×175 and ×80, respectively).*

PLATE II



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The 10-day-old larvae differed little from the six- and eight-day stages. They were not coiled as much as the younger larvae, except when they were embedded in the deeper tissues. Motility was still very slight. The only morphological change was a hollowing out of the proximal portion of the intestine.

Discussion

The results of the experiments showed that the egg hatches in the upper part of the small bowel. This may occur as soon as half an hour after ingestion. That hatching does not occur in the stomach was demonstrated by the recovery of unhatched eggs in this organ at the same time as larvae and unhatched eggs were found in the intestine. These findings do not agree with those of Tochihara (12), who reported that hatching occurred in the stomach as well as in the intestine. It is possible that in his experiments a regurgitation of the duodenal contents carried the larvae back into the stomach. Matsubayashi (7) found that hatching of *T. vulpis* eggs always occurred in the intestine of rats. From the studies on the extracorporeal hatching of the eggs (Miller (8)), in which it was demonstrated that hatching never occurred in the gastric juice, it is not likely that hatching occurs in the stomach.

Hatching usually takes place in the duodenum or upper half of the jejunum, as the majority of newly hatched larvae are usually found in those areas. However, it is not unlikely that eggs may hatch at lower levels of the intestine, but probably never below the upper half of the ileum.

Following the escape of the larvae from the egg shells, a large percentage lose their viability and are carried out of the body in the faeces. This conclusion has been reached as a result of the large numbers of dead and dying larvae recovered in the 1-, 5-, and 10-hr. infections. Within 24 hr. some of the larvae have worked their way deeply into the mucosa of the bowel, usually through the crypts of Lieberkühn. Others remain on the surface and are later carried, probably by the peristaltic movements of the gut, into the lower levels of the small bowel and even to the large bowel, as has been demonstrated by the recovery of actively motile larvae at these levels in the second-, fourth-

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PLATE III. FIGS. 15 to 21. *Eight-day-old larva in situ.*

FIGS. 15 and 16. *Larva lying coiled at the base of a crypt of Lieberkuhn, the gland cells of which it has destroyed. Note dark staining cellular detritus (×80 and ×175, respectively).*

FIG. 17. *Coiled larva at base of crypt. Note destruction of glandular tissue and dark-staining cellular detritus (×175).*

FIG. 18. *Larvae lying coiled up at the base of a crypt of Lieberkuhn. Note expansion of crypt lumen and thinning of glandular wall, but relatively little tissue destruction (×175).*

FIGS. 19 and 20. *Note dense staining tissue detritus at either end of larva (×80 and ×175, respectively).*

FIG. 21. *Note the relative absence of tissue destruction (×80).*

and sixth-day infections, but not in the earlier infections. However, it seems likely that of the larvae carried down, only those that work their way below the surface of the mucosa survive, since in these infections healthy, living larvae were never recovered from the lumen mucus.

From the second to the fourth day the larvae become sluggish and assume a coiled position. The six-day larvae are even more sluggish and for the most part are still tightly coiled. A study of tissue sections of the jejunum has revealed numerous larvae throughout the deeper two-thirds of the mucosa, lying in the crypts of Lieberkühn and in the stroma. As suggested earlier there is some evidence that some of the larvae start to migrate back to the lumen as early as the sixth day after infection. Larvae are never seen below the muscularis mucosae. The eight-day larvae resemble those of the sixth-day and are concentrated at the base of the crypts and frequently in the stroma a short distance above the muscularis mucosae. The results obtained with the 10th-day infection suggest that at this time the majority of the larvae start migrating out of the mucosa into the lumen of the gut and are passively carried down to the large bowel, where later development occurs. At this period the majority of the larvae were found in the lumen of the lower third of the ileum, probably indicating a passive transfer to the large bowel. Although the migration of the larvae from the upper parts of the gut does not occur during the first eight days of the infection, until later infections are studied it cannot be definitely stated that the larvae always migrate to the lower levels of the bowel as early as the 10th day. These results are not in accord with those of Hasegawa (4) who reported that the larvae first appeared in the caecum 70 hr. after infection, and the number then gradually increased. It is possible that Hasegawa mistook the appearance in the caecum of a few larvae accidentally carried down by peristaltic action for the true migration of the larvae from the small intestine. It is not likely that larvae enter, leave, and re-enter the mucosa for a period of six to nine days, as suggested by Matsubayashi (7) in his experiments with *T. vulpis* in rats, because the larvae are very sluggish from the fourth day onward. The fact that he worked with an abnormal host may explain his results.

There is very little increase in size of the larvae during the first four days. The sixth-day larvae, however, show a marked increase in thickness, and demonstrate for the first time a well-defined oesophagus and intestine. The 10th-day larva differs from the sixth-day form only in a widening out of the lumen of the intestine. In figuring the newly hatched larvae of *Trichocephalus crenatus*, Alicata (1) divides the gut into oesophagus and intestine. The present studies indicate that his conception is probably erroneous, and that what he refers to as intestine is in reality the still undifferentiated alimentary tract. The portion he labels "oesophagus" is a thinner, anterior portion of the oesophagus.

No moulting of the larvae was observed from the first to the 10th day of development, nor was a moult of the larva seen within the egg. As there is little increase in the size of the larvae during the first 10 days, it is likely that a moult does not occur until later.

The results of the present experiments suggest that, in disagreement with Neshi (9), but in agreement with Fülleborn (3) and Hasegawa (4), and others, there is no larval lung migration in the life cycle of *T. vulpis.*

The mechanical damage caused in the host by the larvae of *T. vulpis* is relatively slight. This is probably due to the fact that the activity of the larvae is, at best, of a weakly muscular type. Furthermore, beginning with the second to the fourth day, the larvae become inactive and remain in this state for a considerable length of time, probably until they migrate down to the large bowel. However, there is some evidence of a lytic destruction caused by secretions of the larvae, as shown by the liquefaction of tissue cells in the immediate vicinity of the parasites. There is possibly some minor cellular destruction of host tissue cells, due to pressure by the larvae as well.

Acknowledgment

The writer wishes to acknowledge with thanks the help of Dr. Ernest C. Faust, Tulane University, under whose direction this work was carried out.

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THE RELATIVE INSECTICIDAL ACTIVITIES OF DDT AND RELATED ORGANIC MOLECULES[1]

By M. D. Proverbs[2] and Frank O. Morrison[3]

Abstract

Impregnated filter paper, and coated glass substrata, as well as direct spraying techniques were employed to test the relative toxicity of several organic molecules related to DDT, as contact insecticides against adult *Drosophila melanogaster*. Anaesthetizing the flies for convenience of handling did not affect observed mortalities. In general both impregnated paper and coated glass (vial) techniques gave reproducible results. The former resulted in an unexplained drop in mortalities above a certain dosage level, the latter gave somewhat more uniform results. Direct spraying was superior when the chemical had a fumigant effect also.

γ, 1,2,3,4,5,6-Hexachlorocyclohexane and the difluoro analogue of DDT, alone out of 18 chemicals tested, exceeded DDT in effectiveness. The γ isomer of 666 exhibited a strong fumigant but also a long residual effect while the difluoro analogue lost toxicity rapidly.

Removal of one or both phenyl chlorine atoms from the DDT molecule, or alteration of their *p-p* positions, or their substitution by any radical other than F, interference with the trihalogen group of DDT, or the introduction of a double bond into the molecule, all seriously affected the insecticidal action of the DDT. Arranged from the most to the least toxic, the halogen analogues of DDT have the following order: fluorine, chlorine, bromine, iodine.

It has long been recognized that the discovery of some law relating the insecticidal activity of organic molecules to their chemical structure would be of untold value to the synthetic insecticide industry. Läuger *et al.* (13) have recently put forth evidence for one theory of such a relationship. Busvine (8), on the other hand, considers that the search has been for a relationship too simple and direct to be possible in such a complex phenomenon as insecticidal activity. The problem can only be solved when the results from innumerable comparative tests using series of related chemicals against various insects are before us. The picture is even then further complicated by the observed results being so largely a function of the methods used that the value of comparison between experiments is questionable (16). The following data are, it is hoped, a small contribution to the necessary mass of evidence that will eventually settle the question of this relationship or lack of relationship between toxicity and chemical structure.

This investigation began under the impetus of wartime needs when in the summer of 1944 the junior author in co-operation with the Department of Genetics, McGill University, began an attempt to rapidly assay the potential insecticidal properties of a number of organic molecules being synthesized by

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[1] *Manuscript received July 19, 1946.*

Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Que. Macdonald College Journal Series No. 217. Based on a thesis submitted to the Faculty of Graduate Studies and Research at McGill by the senior author, in partial fulfilment of the requirements for the degree of Master of Science.

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the National Research Council Chemists at McGill. The senior author followed up this comparative testing during 1944-45 and 1945-46. Meanwhile Dr. H. C. Browning and Dr. Fraser, Department of Genetics, carried on similar research at McGill. All the investigators were in constant communication. This report, however, records only the work done at Macdonald College, though reference is sometimes made to ideas originated by the other workers and corroboratory results obtained by them.

Methods and Apparatus Employed

Rearing and Handling the Test Animal

All tests reported were conducted using adult *Drosophila melanogaster* of uniform age as the test animal. A potato–yeast medium (14) containing one Royal Yeast cake per 227 gm. of boiled potatoes was used throughout. The rearing cages and technique were those of Stultz (20). During the earlier experiments the flies were manipulated by the method of adapters (16). As the work progressed it was found convenient to anaesthetize the flies for counting.

All the flies of one population were blown into a collecting jar (large candy jar) and a pad of absorbent cotton moistened with 50% ether and 50% ethyl alcohol (95%) placed over the jar mouth where it was held in place by the screw top. The jar was occasionally tapped until all flies were 'down'. The population was then divided among two or three pint milk bottles, stoppered with dry cotton. Larger numbers of flies in one bottle caused moisture to condense on the glass and insects stuck to the bottle. About 600 flies were removed at a time to still another milk bottle where they were subjected to more anaesthesia until all were completely inactive, then poured onto a sheet of white paper from which they were counted into the test cages (shell vials or glass cylinders) with a small camel's hair brush.

Conducting the Tests

(*a*). *By the Impregnated Paper Technique*

The method of exposing flies to a filter paper substratum impregnated with chemical, in order to test the toxicity of that chemical, has been described in the literature (17).

Five or more dosage levels of the chemical under test were used along with an equal number of DDT dosages. The DDT used was a recrystallized product from the Shawinigan Chemical Co. Dosages were chosen to give a wide range of mortalities. Ten vials lined with impregnated paper and loaded with 15 flies each were used for each dosage, on each of seven or eight different days. After each test the vials were cleaned by boiling them in a solution of concentrated sulphuric acid and sodium dichromate, washed in hot water and finally dried in an oven. Chemicals, which when tested in this fashion showed an appreciable loss of effectiveness over a period of two weeks after impregnation, were compared by using 40 to 50 vials of flies at each dosage and completing the tests on the same day on which the papers were prepared or on the day following.

Long term residual toxicity was measured by testing, at successive intervals, papers all impregnated on the same date.

(*b*). *By the Coated Vial Technique*

Dr. Browning (7) reported very satisfactory comparisons using the glass surface itself as a test substratum. At Macdonald College this was effected by using the same size of shell vials as were used with the paper technique. The vials were filled to the brim with acetone solutions of the test chemicals, then immediately emptied and inverted into a wire basket where they were left to drain and dry. Dried vials were stored in cardboard containers in the dark. The procedure in testing was then exactly the same as for the paper technique.

(*c*). *By Spraying the Flies Directly*

Spraying of the flies in cotton covered cylindrical cages was carried out using the continuous spraying technique of McLeod (14) with the atomizer adjusted to deliver 1 ml. of spray in 15 sec. The procedure was identical with McLeod's except that 5% molasses was substituted for 5% honey on the food plugs. Mortality counts were made after 24 hr.

Comparison of Techniques

Experiments Conducted

(*a*). *Flies Handled with and without Anaesthesia*

The handling of flies under anaesthesia made the exact counting of numbers possible, eliminated much apparatus, and eliminated possible selection of more light responsive individuals. It seemed possible, however, that the anaesthetic might alter the biological response. This possibility was tested by exposing flies handled in each manner to DDT coated vials. Five dosage levels were tested each day by each method, the experiment being repeated on seven different days. The observed percentage mortalities are given in Table I*a*, and the probit–log–dosage regression lines for both sets of data in Fig. 1. The same data were analysed by the method of analysis of variance, using angular conversion values (5, 6) and the results of this analysis are tabulated in Table I*b*.

(*b*). *Flies Introduced into Test Cages Before and After Recovery from the Anaesthetic*

Not all anaesthetized flies recover simultaneously. Thus, if the flies are placed in the vials while still inactive there is a possibility that the first to revive may remove much of the coating from a glass surface. If this were the case the flies in any one sample would not all be exposed to equal dosages. Dr. Browning in correspondence suggested this as an explanation of mortality variation from vial to vial at the same dosage. The following experiment was designed to test this theory.

A fly population was anaesthetized, then divided into two lots. One lot was used to stock DDT coated vials, the other group was counted out into clean shell vials. When the flies in the clean vials were all active they were transferred to coated vials by inverting one vial over the other and gently tapping the flies down. Fifty vials at each of two dosages were used for each method. . The mortalities observed are recorded in Table II.

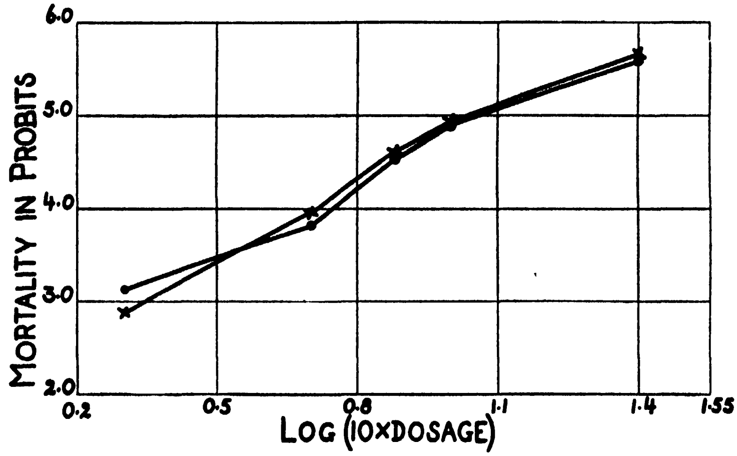


FIG. 1. *Probit–log–dosage curves for flies exposed to DDT when handled with and without anaesthetic.*
Handled with anaesthetic: ×——————×
Handled without anaesthetic: ●——————●

(*c*). *Flies Exposed to Impregnated Papers and Coated Vials*

An experiment was conducted to determine the sensitivity of the impregnated paper technique. Five different samples of DDT were used: (1) prepared in the McGill Chemistry Laboratory, (2) a crystalline sample from the Shawinigan Chemical Co., (3) a crystalline sample from the Geigy Co., (4) a crude sample from a pilot plant, (5) a crude sample prepared by a chemist at Macdonald College. The observed mortalities and regression lines are given in Tables III*a* and III*b* and Figs. 3 and 4.

A direct comparison of the paper and coated vial techniques was made using seven concentrations of DDT. Mortality counts were made on all replications after 24 hr., the first four replications, however, were also counted after 18 hr. to compare the rapidity of action. Tables IV*a* and IV*b* and Figs. 2, 5, 7, and 8 contain the observed results.

A peculiar drop in mortality was observed by the paper technique in the above test, as the dosage was increased from 3 to 10 mgm. of DDT per 10 ml. of acetone. To preclude possibility of an error the complete experiment was repeated with counts made only after 24 hr. The results are shown in Table V and Fig. 6.

The possibility that impregnating solutions might be exhausted by differential adsorption of the solute (DDT) onto filter paper was tested by successively impregnating two batches of papers with the same DDT solution (20 mgm. of DDT in 40 ml. of acetone) and comparatively testing papers from the two batches (Table VI).

DDT has a contact and stomach action but no fumigant action. Hexachlorocyclohexane has a known fumigant action. The paper and coated vial techniques were compared using the latter toxicant. Two dosages were used on three different days. The mortalities observed are recorded in Table VII.

(*d*). *Flies Exposed to Direct Sprays*

Flies confined in cylindrical glass cages with cotton ends were, during later comparisons, sprayed directly with a suspension of DDT prepared by adding an alcoholic solution to water. The results are given in Table VIII*a* and Fig. 9. Though the experiments were carried out at a different time than the paper and coated vial tests, some comparisons of this spraying technique with the paper and coated vial techniques are possible.

Discussion of Results

(*a*). *Flies Handled with and without Anaesthesia*

Examination of Table I*b* shows that there was no statistically significant difference between the mean mortalities observed using anaesthetized and non-anaesthetized flies. Expressed in degrees the difference between the experiment means for the two methods was 1.07. A difference of at least 1.62 would be necessary for significance. If the physiology of *Drosophila* is upset by anaesthetization, it was not reflected in the observed mortalities from exposure to DDT. It was assumed that this would hold true for other chemicals tested.

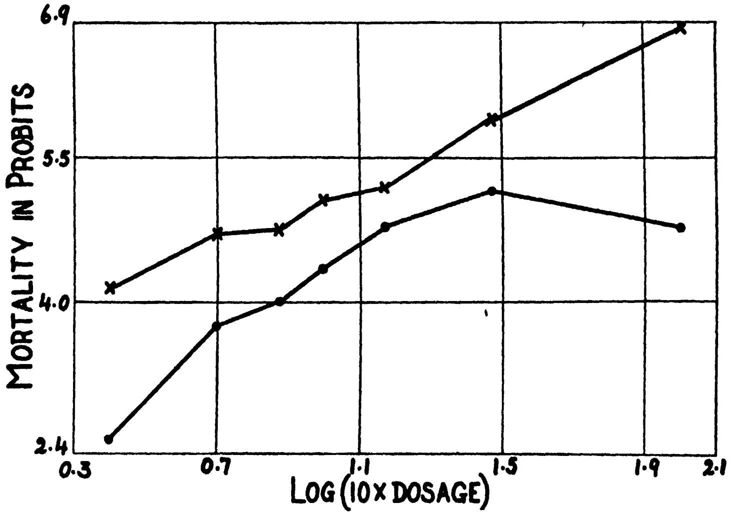


FIG. 2. *Probit-log-dosage curves for flies exposed to DDT by the impregnated paper and coated vial techniques (18-hr. exposure period).*

Impregnated papers: ●————————●

Coated vials: X————————X

(*b*). *Flies Introduced into Test Cages Before and After Recovery from the Anaesthetic*

The mortalities shown in Table II do not show any difference when inactive flies were allowed to recover in the test vials or were not introduced until active. No wider range of vial to vial mortality occurred with either method. As far as the evidence of this limited test goes, it lends no support to the suggested possibility that flies reviving first may deplete the available DDT dosage on a limited glass substratum.

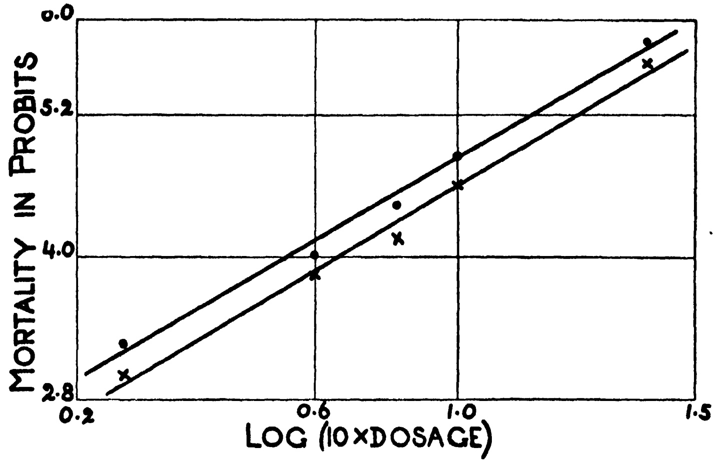


FIG. 3. *Probit–log–dosage regression lines fitted by eye for different samples of DDT.*
McGill (refined sample): ●————●
Geigy (refined sample): ×————×

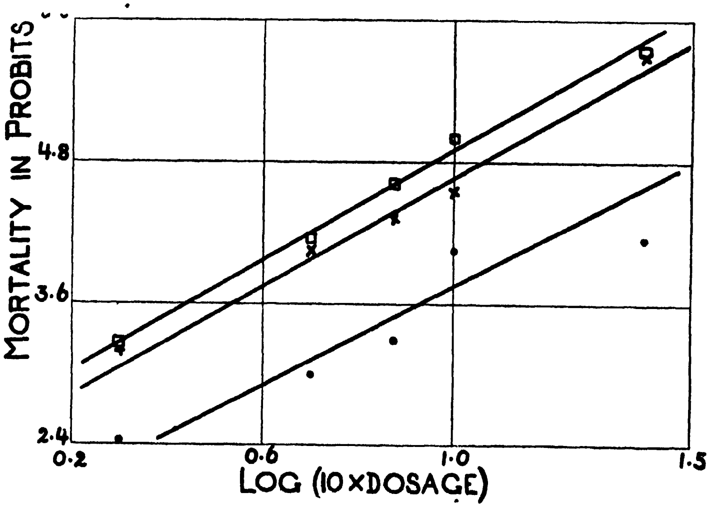


FIG. 4. *Probit–log–dosage regression lines fitted by eye for different samples of DDT.*
Shawinigan (refined sample): □————□
Pilot Plant (crude sample): ×————×
Macdonald (crude sample): ●————●

(*c*). *Flies Exposed to Impregnated Papers and Coated Vials*

The impregnated paper technique was not sufficiently sensitive to reveal any statistically significant difference between DDT samples from different sources or of different degrees of refinement except in the case of one extremely crude oily product produced at the College with this object in mind (Table III*b*). The very fact that the probit-log-dosage curves for the samples

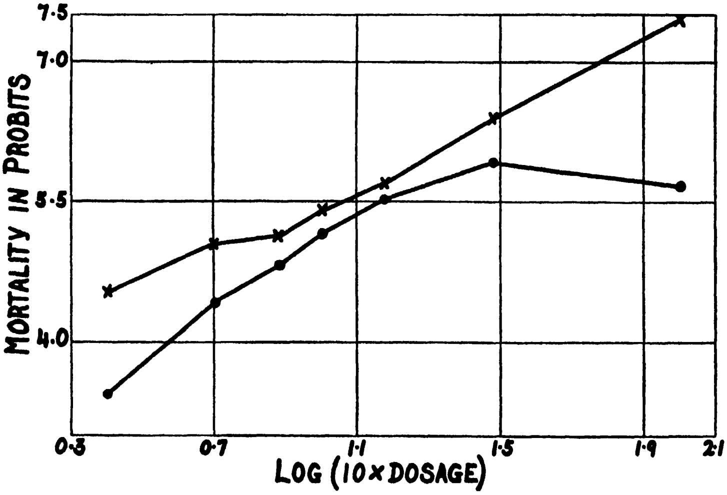


FIG. 5. *Probit-log-dosage curves for flies exposed to DDT by the impregnated paper and coated vial techniques (24-hr. exposure period).*

Impregnated papers: ●————————●
Coated vials: ×————————×

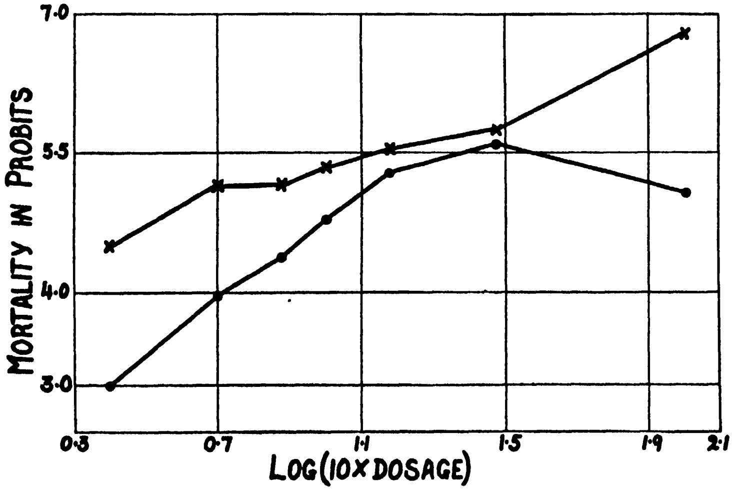


FIG. 6. *Probit-log-dosage curves for flies exposed to DDT by the impregnated paper and coated vial techniques (24-hr. exposure period).*

Impregnated papers: ●————————●
Coated vials: ×————————×

tested are so nearly identical speaks well for the reproducibility of results using this technique.

The data in Tables IV*a*, IV*b*, and V leave no doubt that vials coated with DDT from a solution of given concentration are more highly toxic than papers impregnated from the same solution. The greater surface area offered by the glass, the physical nature of the glass surface making the entire deposit more readily available (1), and the possibility that the deposit on the glass

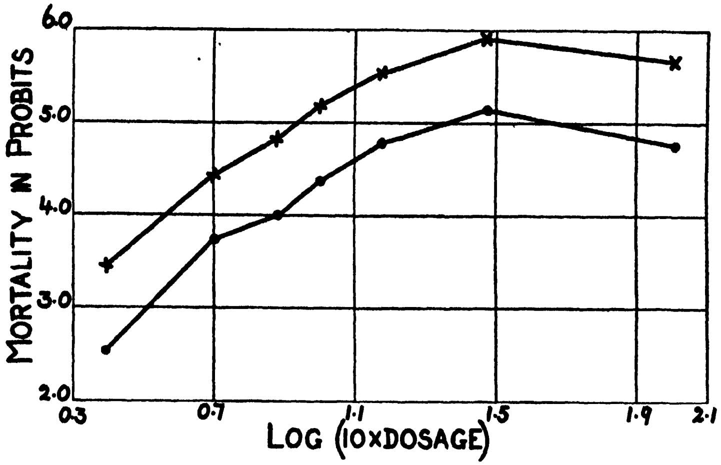


FIG. 7. *Probit–log–dosage curves for flies exposed to DDT by the impregnated paper technique at 18- and 24-hr. exposure periods.*

18-Hr. exposure period: ●————●

24-Hr. exposure period: ×————×

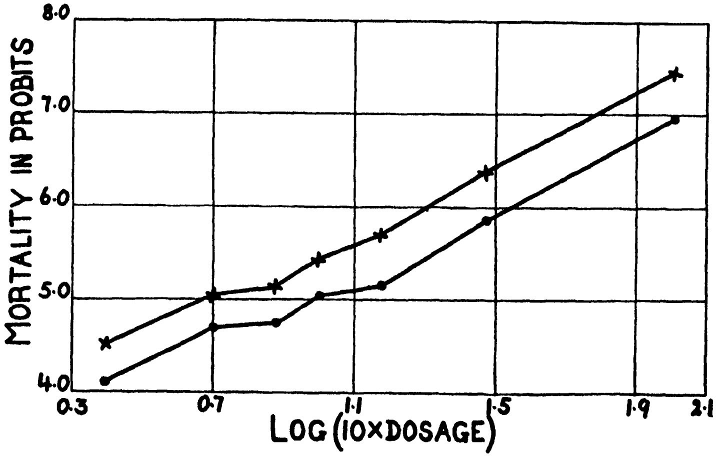


FIG. 8. *Probit–log–dosage curves for flies exposed to DDT by the coated vial technique at 18- and 24-hr. exposure periods.*

18-Hr. exposure period: ●————●

24-Hr. exposure period: ×————×

where contact was made with the food plugs may have been imbibed and served as a stomach poison may all help to account for this result.

The kill by both techniques was lower at the end of 18 hr. than after 24 hr., but the resulting toxicity curves are obviously parallel (Figs. 7 and 8) indicating that results with either exposure would be validly comparable. At the end of 18 hr. many flies were still dying so that slight differences in the exactness of the exposure period would greatly affect the observed mortalities. No logical explanation has as yet occurred to the authors for the curious dropping off of observed mortalities with increased dosage observed between the dosages of 3 and 10 mgm. of DDT per 10 ml. of acetone (Tables IV*a* and IV*b*) using the impregnated paper technique. The chance that an error might have occurred in preparation of the solutions was eliminated by the repetition of the experiment (Table V, Fig. 6). If when cellulose paper is immersed in a dilute DDT solution the DDT itself were adsorbed more rapidly than the solvent, the first papers immersed would acquire a larger amount of DDT (i.e. a higher dosage) than those immersed last. Such a differential adsorption rate has been postulated as occurring when the chitin of insect cuticula is immersed in dilute DDT solutions (18). Some low dosage papers in a sample from which a high mortality was expected might greatly lower the average observed mortality. No evidence, however, of such differential adsorption is afforded by mortalities from batches of paper successively treated with the same solution (Table VI). There remains the possibility that solutions of different concentration deposit crystals of different sizes and thus affect mortality (1).

Combining the data from several experiments in which the same dosages of DDT were used by the paper technique results in average mortalities based on 6000 flies at each concentration (Table XV*a*). Similar combined data involving 3000 flies per dosage by the vial technique are given in Table XV*b*. The dosages used in the paper technique were below 3 mgm. of DDT per 10 ml. of solvent and hence the drop in mortality at high dosages is not shown. Examination of the calculated probit–log–dosage regression lines (Fig. 9) for these sets of combined data reveal a higher Chi^2 value (185) for the impregnated paper than for the coated vial technique. Part of the larger value for the former is accountable on the basis of the larger number of flies involved (9, 14, 16) but nevertheless the coated vial technique seems to yield data in greater conformity with the straight regression line theory of Bliss (2, 3, 4). The slope of the paper technique line is steeper indicating a greater rate of increase in mortality with increase in dosage up to the dosages here tested.

A chemical having a fumigant as well as a contact effect, such as hexachlorocyclohexane, gave, as one would expect, much higher mortalities when tested on papers than when deposited on the glass of the vials (Table VII). Since this is opposite to the results obtained when DDT was used it would seem to suggest that the papers carry more of the chemical than vials treated with equally concentrated solutions but less of it is available for contact action on the insect. Neither technique is satisfactory for assaying the contact effect when a fumigant effect is also present.

Chemicals that gradually lose toxicity when exposed in thin layers must be assayed on the day the surfaces are prepared if either technique is used.

The impregnated paper technique is better suited to testing residual toxicity over a long period as when vials are used storage of large numbers becomes a problem. Coated vials can be more quickly prepared than papers and allow greater ease of counting mortality. With papers flies may become trapped between the paper and glass and die of starvation or injury. The coated vials, as previously mentioned, may encourage some feeding on the toxicant at the margin of the food plugs.

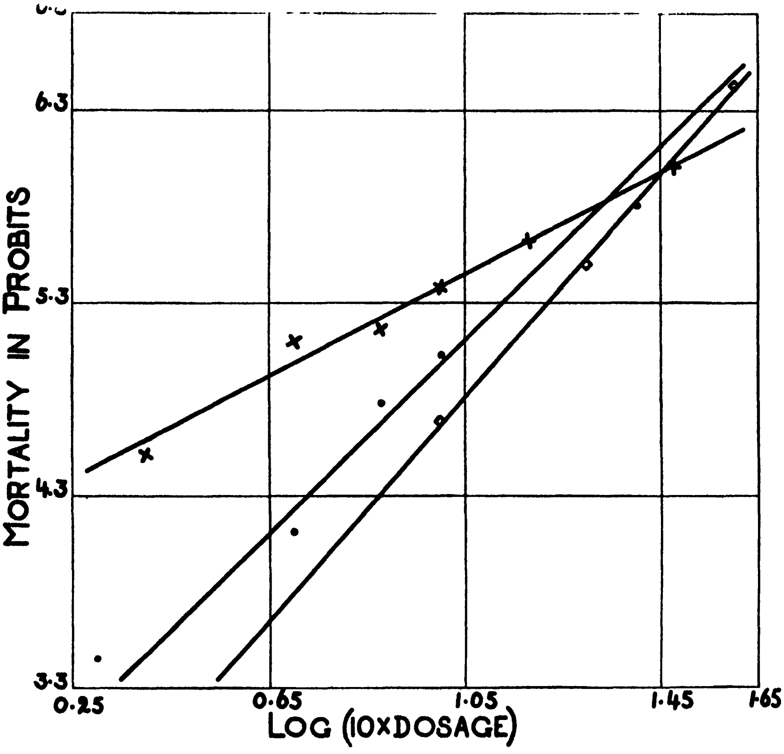


FIG. 9. *Probit–log–dosage regression lines for flies exposed to DDT by the coated vial, impregnated paper, and spraying techniques.*

DDT coated vials: ×————×
Regression line formula = $Y = 5.268 + 1.330\ (X + 0.915)$.
Chi^2 = 30.994.
Variance for position = $V\ (a) = 0.0010$.
Variance for slope = $V\ (b) = 0.0091$.

DDT impregnated papers: ●————●
$Y = 4.784 + 2.252\ (X + 0.922)$.
Chi^2 = 185.07.
$V\ (a) = 0.0035$.
$V\ (b) = 0.0337$.

DDT spray: □————□
Line fitted by eye.

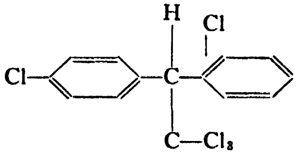
(*d*). *Flies Exposed to Direct Sprays as Compared with Those Exposed Otherwise*

Examination of Fig. 9 reveals the probit–log–dosage regression line obtained by spraying the flies directly with a DDT suspension to be reasonably parallel with the line for impregnated papers (combined data) and hence somewhat steeper than that obtained with coated vials. It must be remembered that direct spraying measures the immediate contact effect (and possible stomach effect if any is imbibed) while the paper and coated vial techniques measure residual contact effect. The open cages and short exposure (one-half hour after spraying before transfer) make direct spraying somewhat more suitable for testing chemicals having fumigant as well as contact effects.

Comparison of Chemicals

Experiments Conducted

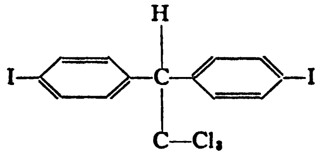
1. 1-(2-Chlorophenyl)-1-(4-chlorophenyl)-2,2,2-trichloroethane (The *o-p* isomer of DDT)



Five dosage levels were used by the paper technique and the test replicated on eight different days. Table IX and Fig. 11 show the average observed mortalities and the calculated probit–log–dosage regression line. DDT was not tested on the same populations but the DDT line based on 6000 flies per dosage (Table XV*a*) has been inserted on the graph for comparison.

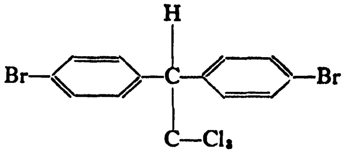
Any possible synergistic effect due to the presence of the *o-p* isomer in commercial DDT was tested using four dosages of DDT alone and in combination with four dosages of its *o-p* isomer. The impregnated paper technique was used. The observed mortalities are given in Table X*a* and the results of an analysis of variance using angular conversion values and aimed at showing up any interaction of the effects of the chemicals used in mixtures appear in Table X*b*.

2. 1,1-Bis-(*p*-iodophenyl)-2,2,2-trichloroethane



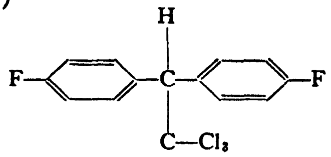
Using the paper technique *Drosophila* was exposed to dosages as high as 500 mgm. per 10 ml. of alcohol, without any mortality being observed.

3. 1,1-Bis-(*p*-bromophenyl)-2,2,2-trichloroethane



Five dosages were used by the impregnated paper technique and the experiment replicated eight times. Table XI and Fig. 13 set forth the results. A single DDT dosage was tested on the same populations and through the point thus obtained a regression line has been drawn parallel to that obtained from the data on 6000 flies per dosage, i.e., the data in Table XV*a*.

4. 1,1-Bis-(*p*-fluorophenyl)-2,2,2-trichloroethane (active principle of German insecticide 'Gix' (12))

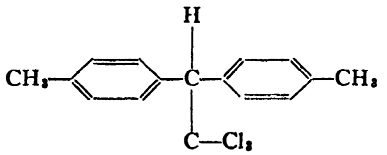


Six dosages were tested in parallel with six dosages of DDT using the coated vial technique (Table XII*a*).

Flies confined in cylinders of wire gauze were placed in 10 vials coated with a dosage of 2 mgm. of this difluoro analogue of DDT per 10 ml. of acetone. Mortality after 24 hr. was only 6.7% although this dosage level killed nearly 100% by contact. The check mortality using wire gauze cylinders in untreated vials was 1.3%.

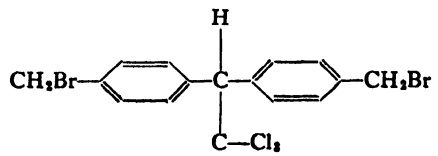
Mortalities observed when three dosages were used and 40 vials employed on the same day at each dosage are shown in Table XII*b* and Fig. 12. DDT was tested in parallel with this series.

5. 1,1-Bis-(*p*-methylphenyl)-2,2,2-trichloroethane



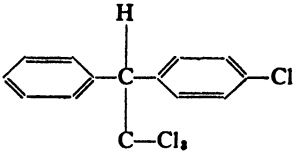
Six dosages of the dimethyl analogue of DDT and a similar number of DDT were tested in parallel by the paper technique (Table XIII, Fig. 14).

6. 1,1-Bis-(*p*-bromomethylphenyl)-2,2,2-trichloroethane



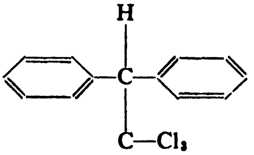
Concentrations as high as 500 mgm. per 10 ml. of alcohol tested by the paper technique gave no mortality.

7. 1-Phenyl-1-*p*-chlorophenyl-2,2,2-trichloroethane



Using the coated vial technique five dosages of this chemical were tested in parallel with five DDT dosages (Table XIV, Fig. 15).

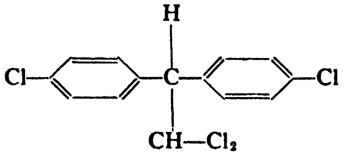
8. 1,1-Diphenyl-2,2,2-trichloroethane



A dosage of 150 mgm. per 10 ml. of acetone by the coated vial technique gave only 5% mortality.

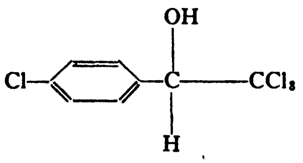
9. 1,1-Bis-(*p*-chlorophenyl)-2,2-dichloroethane (DDD)

The chemical tested was a commercial sample supplied by the Rohm and Haas Co.



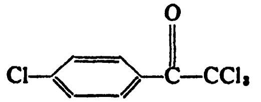
Preliminary tests indicated a loss of effectiveness over a period of time. Five dosages were tested in parallel with five dosages of DDT using the coated vial technique and exposing 40 vials at each dosage, 24 hr. after coating the vials (Table VIII*b* and Fig. 17).

10. 1-(4-Chlorophenyl)-2,2,2-trichloroethanol ('Carbinol DDT')



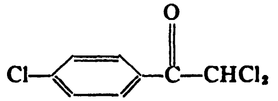
The toxicity of exposed surfaces fell off rapidly so testing was completed on one day. Five dosages were compared with an equal number of DDT dosages using the coated vial technique and 50 vials for each dosage (Table XVI*b* and Fig. 19).

11. *p*-Chloro-*α*-trichloroacetophenone



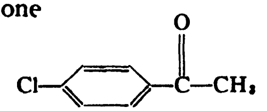
This compound lost toxicity rapidly on exposure. Five dosages were tested in parallel with DDT using 50 coated vials for each dosage of each chemical (Tables XVI*a* and XVI*c* and Fig. 18).

12. *p*-Chloro-*α*-dichloroacetophenone



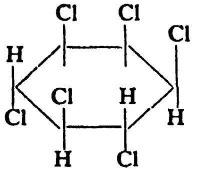
This chemical has an irritating odour and flies exposed to concentrated fumes succumb readily. Impregnated papers were used to measure the loss of toxicity over a period of three months (Table XVII).

13. *p*-Chloroacetophenone



This material also has an acrid odour and toxic fumes. The long term residual effect was tested by using impregnated papers over a period of three months (Table XVII).

14. The γ-isomer of hexachlorocyclohexane (γ isomer of 666, or Gammexane)



Using wire gauze cylinders containing flies in vials lined with impregnated paper (0.25 mgm. of 666 per 10 ml. of acetone), 150 flies (10 vials) were tested on each of three successive days. After 24 hr. the mortalities averaged 20% but would have been much greater with a longer exposure period (10, 19). Check mortalities were only 1%.

Eight dosages were prepared by dissolving different amounts of 666 in 5 ml. lots of acetone and diluting each such solution with 45 ml. of distilled water. At the concentrations used precipitation in the water did not occur. The eight dosages were tested by the direct spraying technique. One DDT dosage was similarly tested on the same populations. The DDT was dissolved in alcohol instead of acetone because solutions in the former solvent, when diluted with water, give a colloidal suspension of the solute, while the DDT from acetone solutions precipitates in flake form when water is added.

Twenty cages of flies were used at each dosage and the experiment replicated four times. Percentage mortalities observed are listed in Table XVIII*a*, and the probit–log–dosage regression line for 666 is given in Fig. 10 along with a DDT line drawn through the determined point and parallel to the line determined for DDT when DDD and DDT were compared.

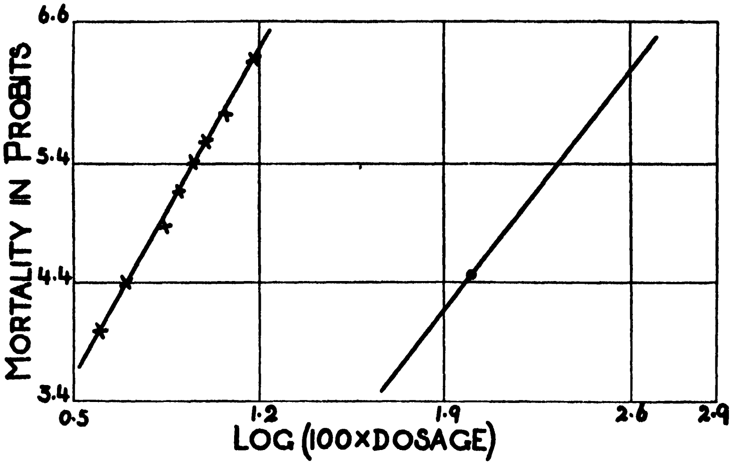
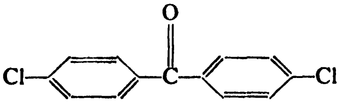


FIG. 10. *Probit–log–dosage regression lines for flies sprayed with 666 and with DDT.*
666 spray: X————X
$Y = 5.154 + 3.926\ (X + 0.899).$
$Chi^2 = 9.1550.$
$V(a) = 0.0003.$
$V(b) = 0.0116.$
DDT spray: ●————●
Line fitted by eye.

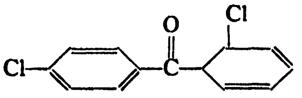
γ-Hexachlorocyclohexane at 0.25 mgm. per 10 ml. of acetone, and later at 0.1 and 0.05 mgm. per 10 ml. was tested in parallel with DDT for residual effect over a period of four months (Table XVIII*b*).

15. *p*-*p*-Dichlorobenzophenone



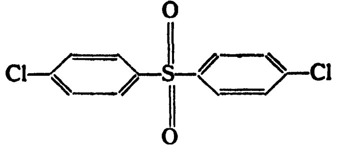
A dosage of 50 mgm. per 10 ml. of acetone was ineffective by the coated vial technique.

16. 2,4′-Dichlorobenzophenone



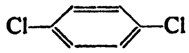
At 50 mgm. per 10 ml. of acetone this material was ineffectively tested by the coated vial technique.

17. Bis-(*p*-chlorophenyl)-sulphone



Results as with Nos. 15 and 16.

18. *p*-Dichlorobenzene



Results as with Nos. 15, 16, and 17.

Discussion of Results

1. *o-p* DDT. The *o-p* isomer of DDT is the major impurity in the commercial product and is hence of great interest. Using Fig. 11 and comparing the dosages required to kill 50% of the population, this chemical, tested by the impregnated paper method was 1/145th as active as DDT. The toxicity curves are definitely parallel suggesting a similar mode of action. This parallelism is so marked that a suspicion arises. The *o-p* isomer used was isolated from a crude DDT and may well have contained some DDT. An amount of 0.25% by weight of DDT would account for the observed

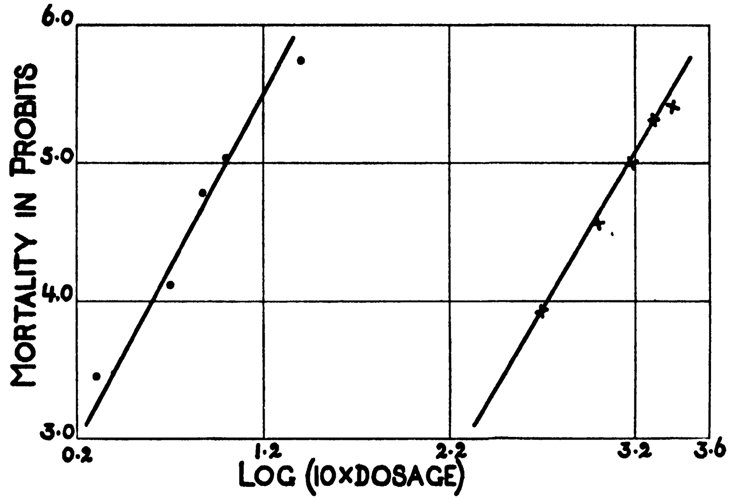


FIG. 11. *Probit–log–dosage regression lines for flies exposed to DDT and to its o-p isomer by the impregnated paper technique.*

DDT impregnated papers: ●————————●
Line fitted by eye.
o-p DDT impregnated papers: ×————•————×
$Y = 4.883 + 2.174\ (X - 13.141)$.
$Chi^2 = 2.8701$.
$V(a) = 0.0002$.
$V(b) = 0.0041$.

toxicity. In correspondence the McGill workers have indicated that a pure sample of the *o-p* isomer was quite inactive. Busvine (8) working with *Cimex lectularius* and *Pediculus humanus* attributes a slight activity to the *o-p* isomer. The history of the sample used is not given. Gersdorff (11) reports DDT to be 53 times as toxic as its *o-p* isomer to houseflies.

Table X*b* shows a complete lack of interaction and hence presumably of synergism, in the action of DDT and of DDT mixtures. Busvine (8) reports similar findings.

2. The di-iodo analogue of DDT. The complete inactivity of this molecule seems an unexpected result and establishes the importance of the chlorine atoms in the *p*-positions on the phenyl rings.

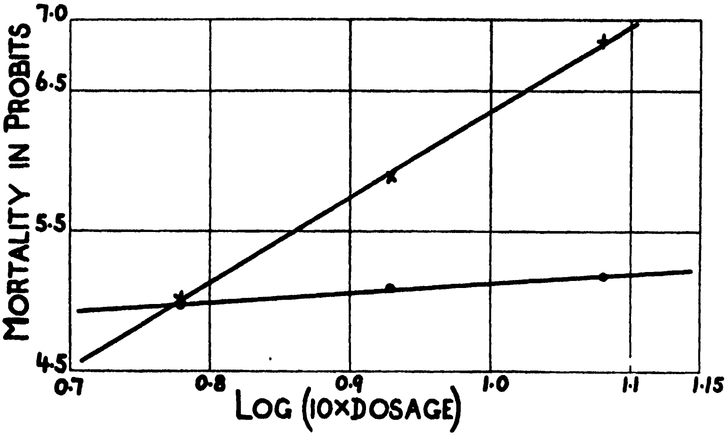


FIG. 12. *Probit–log–dosage regression lines for flies exposed to DDT and to its difluoro analogue by the coated vial technique.*
DDT: ●————●
Difluoro analogue: ×————×

3. The dibromo analogue of DDT. The regression lines (Fig. 13) give a toxicity of 1/26 relative to DDT, for this analogue. The curious break in the dibromo line might indicate a change in mode of action or the presence of a second toxicant such as DDT in small amounts.

4. The difluoro analogue of DDT. Tested 20 hr. after coating the vials the difluoro analogue was nine times as toxic as DDT (Fig. 12). This places the order of toxicity of the halogen analogues as fluorine, chlorine, bromine, and iodine. Volatility, however, here adds a complicating factor in estimating test results. Toxicity was lost rapidly (Table XII*a*). The slope of the regression line for difluoro DDT is much steeper than that for DDT so that comparisons will depend on the level of mortality at which they are made.

The fumes of this chemical were alone slightly toxic as has already been stated.

Since difluoro DDT appears to have been the active principle of the German wartime insecticide 'Gix' this analogue is of especial interest.

5. The dimethyl analogue of DDT. The extremely low toxicity of this analogue is of interest in view of the high toxicity reported for the methoxy analogue (8). The latter material was not tested during this work.

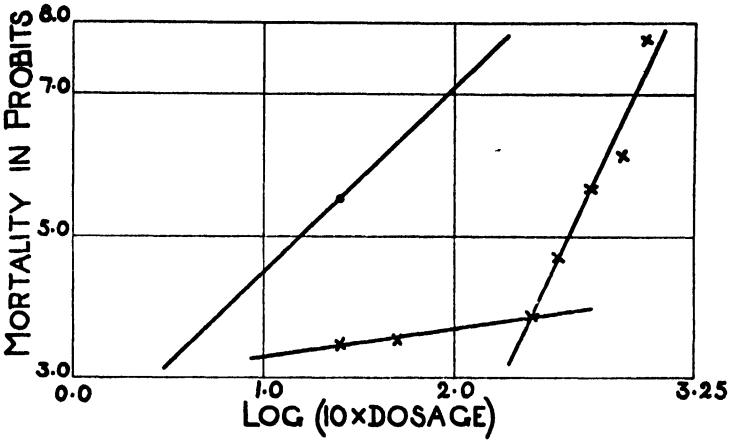


FIG. 13. *Probit-log-dosage regression lines for flies exposed to DDT and to its dibromo analogue by the impregnated paper technique.*

DDT: ●——————●

Dibromo analogue: ×——————×

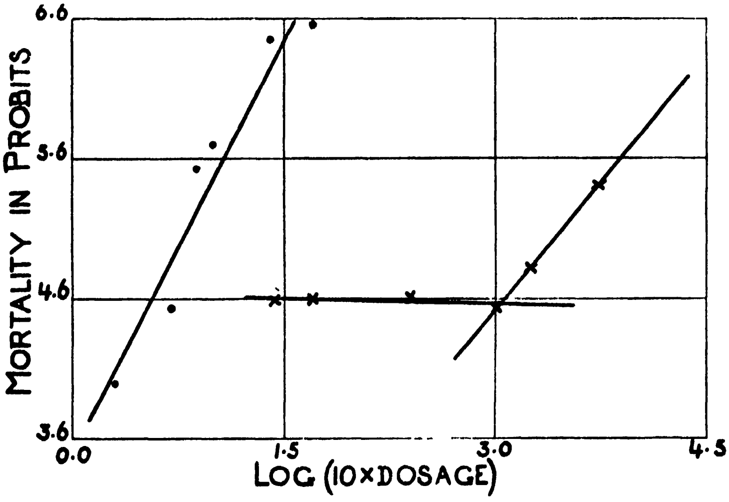


FIG. 14. *Probit-log-dosage regression lines for flies exposed to DDT and to its dimethyl analogue by the impregnated paper technique.*

DDT: ●——————●

Line fitted by eye.

Dimethyl analogue of DDT: ×——————×

6. The dibromomethyl analogue of DDT. The addition of the methyl groups resulted in a complete loss of the slight activity registered by the dibromo analogue.

7. 1-Phenyl-1-*p*-chlorophenyl-2,2,2-trichloroethane. This molecule is reported as a common impurity in commercial DDT. Calculated from Fig. 15, it has a toxicity of 1/90 that of DDT at the 50% kill point. The slope of the line is less steep than for DDT.

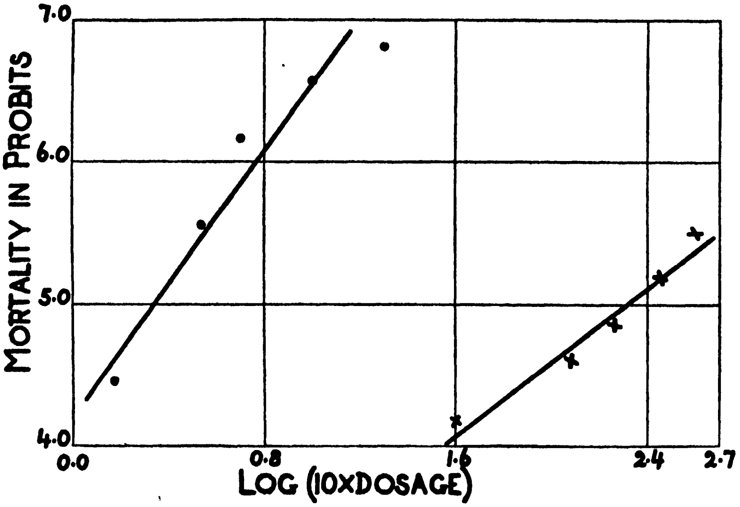


FIG. 15. *Probit–log–dosage regression lines for flies exposed to DDT and to 1-phenyl-1-p-chlorophenyl-2,2,2-trichloroethane by the coated vial technique.*

DDT coated vials: ●——————●

1-Phenyl-1-p-chlorophenyl-2,2,2-trichloroethane: ×——————×

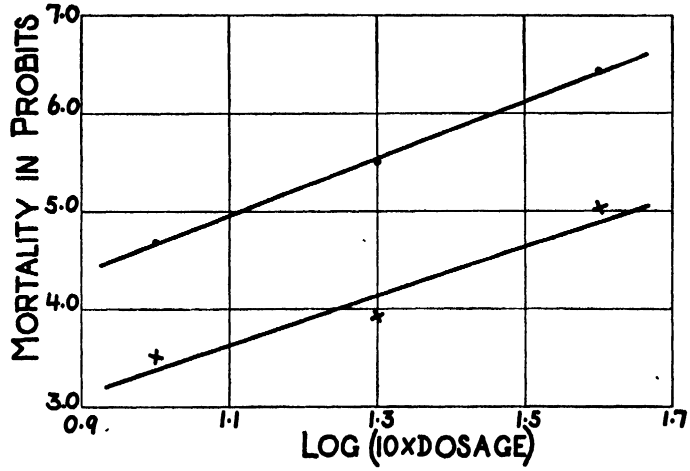


FIG. 16. *Probit–log–dosage regression lines for flies sprayed with DDT and with DDD.*

DDT: ●——————●

DDD: ×——————×

8. 1,1-Diphenyl-2,2,2-trichloroethane. The non-toxicity or low toxicity of this compound as tested here and by Busvine (8) gives further evidence of the importance of the chlorine atoms in the phenyl rings.

9. DDD. A loss of toxicity on exposure of this material was established in preliminary tests. The toxicity relative to DDT at the median lethal dosage, as established in Fig. 17, is 1/17. Tested by the spray technique, however (Fig. 16), it was one-third as toxic, bearing out an earlier conclusion of the junior author (16) that observed comparative toxicities are as largely functions of the technique used as of the materials under test. Busvine (8) rated DDD very toxic to lice and bedbugs, being exceeded in toxicity by only two compounds, dimethoxy DDT and DDT. The commercial manufacture and advertising of DDD with a view to its sale as an insecticide makes it a highly interesting molecule.

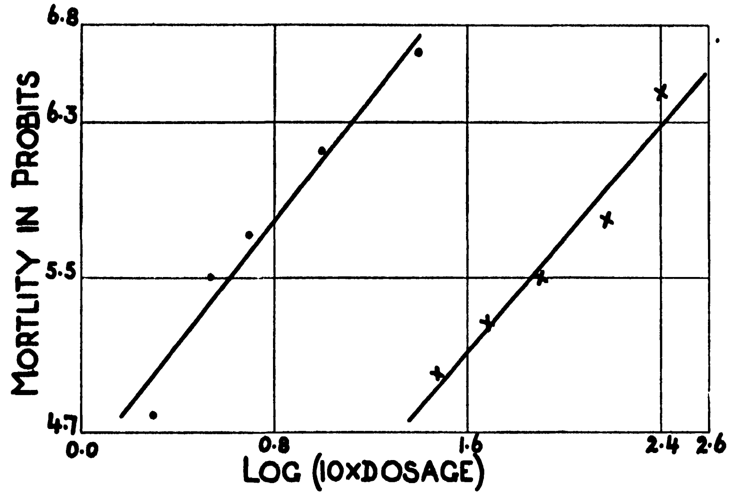


FIG. 17. *Probit–log–dosage regression lines for flies exposed to DDT and to DDD by the coated vial technique.*

DDT: ●——————●
Line fitted by eye.
DDD: ×——————×
$Y = 5.499 + 1.430\ (X + 1.862).$
$Chi^2 = 30.6501.$
$V\ (a) = 0.0033.$
$V\ (b) = 0.0334.$

10. Carbinol DDT. The toxic effect of carbinol DDT is lost rapidly on exposure (Table XVI*a*). Tested 12 hr. after coating the vials (Fig. 19), it appears to be 1/25 as toxic as DDT making it one of the more toxic materials tested. The toxicity curve is almost parallel to that of DDT. Busvine (8) records carbinol DDT as slightly toxic to lice.

11. *p*-Chloro-α-trichloroacetophenone. Marked by its rapid loss of toxicity, this compound was nevertheless 1/40 as active as DDT when tested 12 hr. after the vials were coated. The toxicity line is again remarkably parallel (Fig. 18) with that for DDT and numerous others of this group of compounds. Non-parallelism was the exception rather than the rule.

12. *p*-Chloro-α-dichloroacetophenone. The toxicity of this molecule was low compared to DDT. No numerical comparison is possible on the tests

made. The acrid fumes were shown to be toxic to insects and the toxicity of impregnated papers fell off strongly over three months especially at the lower dosage tested (Table XVII).

13. *p*-Chloroacetophenone. The nature and action of this chemical was essentially the same as that of No. 12 (Table XVII).

14. The γ isomer of hexachlorocyclohexane.

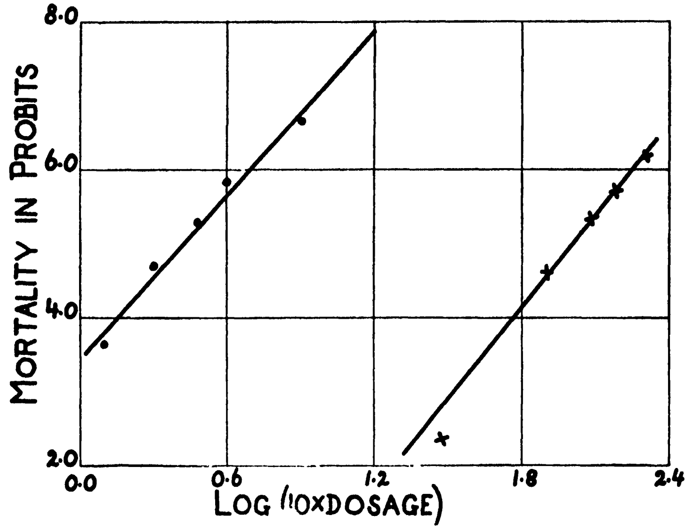


FIG. 18. *Probit–log–dosage regression lines for flies exposed to DDT and to p-chloro-α-trichloroacetophenone by the coated vial technique.*

DDT: ●————●
Line fitted by eye.
p-chloro-α-trichloroacetophenone: ×————×
$Y = 5.372 + 4.112\,(X + 2.095)$.
$Chi^2 = 6.1631$.
$V(a) = 0.0014$.
$V(b) = 0.0577$.

A direct spraying technique was used to minimize the fumigant effect (10, 19) of this chemical in comparisons. Estimated from Fig. 10 Gammexane was 20 times as toxic as DDT at the 50% mortality point. The toxicity line for Gammexane is extremely steep so that comparisons will depend on the point selected for comparison. Barnes (1) testing Gammexane against bedbugs found it to be more effective than 10 times the dosage of DDT, but five months later the residue was non-toxic whereas the DDT residue remained highly active. Attempts made by the authors to measure the loss of activity of Gammexane on impregnated papers failed to reveal any drop in toxicity after four months (Table XVIII*b*) even with a dosage as low as 0.25 mgm. per 10 ml. of acetone. Unfortunately, the dosage chosen gave 100% kill on all tests and may have been so high that the loss did not register.

The phenomenal toxicity of this molecule is especially interesting in view of its production as a commercial insecticide.

15. p-p-Dichlorobenzophenone.
16. 2,4′-Dichlorobenzophenone.
17. Bis-(p-chlorophenyl)-sulphone.
18. p-Dichlorobenzene.

Nos. 15, 16, 17, and 18 were ineffective in comparatively large doses. This result is of some interest in connection with No. 17 (bis-(p-chlorophenyl)-sulphone, which is another common impurity in DDT) and is stated by Läuger *et al.* (13) to be a highly toxic stomach poison.

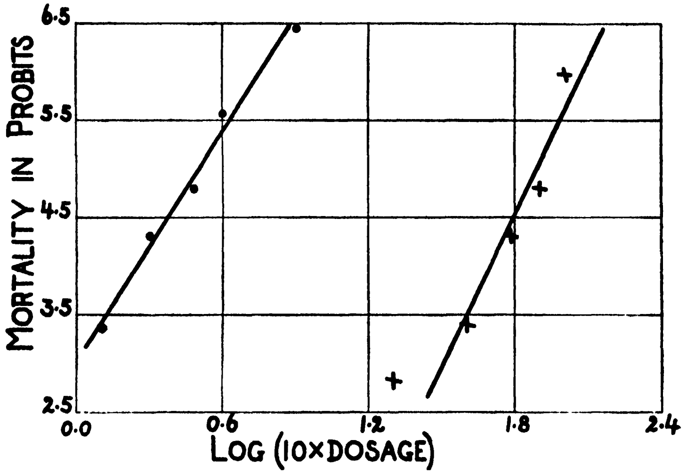


FIG. 19. *Probit–log–dosage regression lines for flies exposed to DDT and to 'carbinol DDT' by the coated vial technique.*

DDT: ●——————●
Line fitted by eye.
'Carbinol DDT': ×——————×
$Y = 4.713 + 5.286\ (\times + 1.830).$
$Chi^2 = 167.664.$
$V\ (a) = 0.0330.$
$V\ (b) = 1.1677.$

General Conclusions

The limited evidence of the data presented here suggests some tentative ideas on the possible relationships between contact insecticidal action, as tested here, and the structure of the organic molecules.

(*a*) Removal of the chlorine from one phenyl group (No. 7) reduced the effectiveness of DDT to 1/90, but removal of the chlorines from both phenyls (No. 8) resulted in a totally inactive compound.

(*b*) Alteration of the p-p arrangement of the chlorine atoms on the phenyl groups, such as in o-p DDT (No. 1) reduced the toxicity to 1/145.

(*c*) Replacement of the two chlorine atoms on the phenyl groups by other halogens shows the order of toxicity of these substitutions to be—fluorine (No. 4), chlorine, bromine (No. 3), iodine (No. 2), but the more highly toxic fluorine analogue unfortunately loses toxicity rapidly on exposure.

(*d*) Replacement of the *p*- substituted chlorines on the phenyl groups by groups other than halogens (No. 5 and No. 6) as far as the tests described here are concerned resulted in greatly lowered toxicities. However, Busvine (8) and Barnes (1) have both found the methoxy analogue of DDT almost as toxic as DDT seeming to indicate that the toxicity of such substitutions may depend entirely on the radicles substituted.

(*e*) Unsaturated bonds (No. 15 and No. 16) appear to reduce the toxicity markedly.

(*f*) Removal of the trihalogen grouping (No. 9, No. 15, No. 16) in so far as it was here attempted resulted in considerable reduction of activity on slight alteration (No. 9) to complete inactivity with complete removal or substitution (15).

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Note: Tables will be found on pages 35-44.

TABLE I*a*

MORTALITIES OF *D. melanogaster* HANDLED WITH AND WITHOUT ANAESTHETIZATION AND EXPOSED TO DDT BY THE IMPREGNATED PAPER TECHNIQUE (10 VIALS FOR EACH DOSAGE, REPLICATED SEVEN TIMES)

—	Anaesthetized flies						Non-anaesthetized flies					
Mgm. DDT per 10 ml. acetone	0.00	0.20	0.50	0.75	1.00	2.50	0.00	0.20	0.50	0.75	1.00	2.50
% Mortality (based on totals)	0.76	2.96	12.8	32.2	41.9	72.1	0.32	1.74	14.88	34.8	43.7	74.4

TABLE I*b*

ANALYSIS OF VARIANCE ON MORTALITIES (CONVERTED TO ANGULAR VALUES) OF *D. melanogaster* HANDLED WITH AND WITHOUT ANAESTHETIZATION AND EXPOSED TO DDT BY THE IMPREGNATED PAPER TECHNIQUE

Sources of variation	Sums of squares	Degrees of freedom	Variance	*F* value	*F* value of 5% point
Days	2964.19	6	496.32	12.4	2.29
Dosages	21,174.64	4	5293.66	132.4	2.56
Handling methods	60.93	1	60.93	1.52	4.00
Error	2319.19	58	39.99	—	—
Total	26,518.95	69	—	—	—

Standard deviation of the difference between two means of 35 variates each = 0.81°.
Means: anaesthetized, 32.31°; non-anaesthetized, 33.72°; difference, 1.41°.

TABLE II

MORTALITIES OF *D. melanogaster* EXPOSED TO DDT BY THE COATED VIAL TECHNIQUE FOLLOWING INTRODUCTION INTO VIALS (1) WHEN UNDER ANAESTHETIC, (2) AFTER RECOVERY (50 VIALS FOR EACH DOSAGE. ENTIRE TEST COMPLETED ON ONE DAY)

—	Introduced when under anaesthetic			Introduced after recovery		
Mgm. DDT per 10 ml. acetone	0.00	0.25	1.00	0.00	0.25	1.00
Average percentage mortality	0.75	50.1	89.8	0.50	50.3	87.6

TABLE IIIa

MORTALITIES OF *D. melanogaster* EXPOSED BY THE IMPREGNATED PAPER TECHNIQUE TO FIVE DDT SAMPLES (10 VIALS FOR EACH DOSAGE, REPLICATED EIGHT TIMES)

Sample No.	Source	Mgm. DDT per 10 ml. alcohol				
		0.20	0.50	0.75	1.0	2.5
		% Mortality (based on totals)				
1. (Refined)	McGill	4.29	16.1	28.4	43.5	79.3
2. (Refined)	Shawinigan	3.86	19.1	34.7	50.0	77.1
3. (Crude)	Pilot Plant	3.49	16.5	24.9	32.5	76.0
4. (Crude)	Macdonald	0.47	2.33	4.28	16.9	19.6
5. (Refined)	Geigy	2.32	12.2	19.9	34.6	73.5

TABLE IIIb

ANALYSIS OF VARIANCE ON MORTALITIES (CONVERTED TO ANGULAR VALUES) OF *D. melanogaster* EXPOSED BY THE IMPREGNATED PAPER TECHNIQUE TO FIVE DDT SAMPLES

Sources of variation	Sums of squares	Degrees of freedom	Variance	*F* value	*F* value of 5% point
Materials	11,853.2794	4	2963.319	31.5	5.63
Dosages	53,120.1806	4	13,280.040	141.3	5.63
Days	6,788.2177	7	969.745	10.3	3.23
Error	17,299.2812	184	94.018	—	—
Total	89,060.9589	199	—	—	—

Standard deviation of the difference between any two 'material' means of 40 variates each = 2.5°.

Difference between 'material' means required for significance = 5°.

'Material' means	*No. 1*	*No. 2*	*No. 3*	*No. 4*	*No. 5*
	31.40	*34.76*	*32.29*	*13.15*	*34.76*

TABLE IVa

MORTALITIES OF *D. melanogaster* EXPOSED TO DDT BY THE COATED VIAL AND THE IMPREGNATED PAPER TECHNIQUES; TO DETERMINE RELATIVE DIFFERENCES IN THE TWO TECHNIQUES (10 VIALS FOR EACH DOSAGE, REPLICATED FOUR TIMES, WITH AN EXPOSURE PERIOD OF 18 HR.)

——	Impregnated paper								Coated vial							
Mgm. DDT per 10 ml. acetone	0.00	0.25	0.50	0.75	1.00	1.50	3.00	10.00	0.00	0.25	0.50	0.75	1.00	1.50	3.00	10.00
Average percentage mortality	0.00	0.67	10.2	15.8	26.7	41.3	56.0	41.0	0.00	19.3	38.3	39.8	51.8	56.5	81.2	96.9

TABLE IV*b*

MORTALITIES OF *D. melanogaster* EXPOSED TO DDT BY THE COATED VIAL AND THE IMPREGNATED PAPER TECHNIQUES; TO DETERMINE RELATIVE DIFFERENCES IN THE TWO TECHNIQUES (10 VIALS FOR EACH DOSAGE, REPLICATED SEVEN TIMES, WITH AN EXPOSURE PERIOD OF 24 HR.)

——	Impregnated paper								Coated vial							
Mgm. DDT per 10 ml. acetone	0.00	0.25	0.50	0.75	1.00	1.50	3.00	10.00	0.00	0.25	0.50	0.75	1.00	1.50	3.00	10.00
Date	Average percentage mortality															
Jan. 23	0.0	3.33	22.0	30.7	48.7	67.4	79.4	66.7	0.0	33 3	46.7	65.3	76.0	85.4	88.7	99.4
Jan. 24	0.0	1.3	6.0	23.3	40.0	50.0	73.4	56 7	0.0	20.7	41.3	52 6	68.7	76.0	90.7	99.4
Jan. 25	0.0	21.3	46.0	63.4	70.7	78.0	93 4	81.4	0.7	44.0	78.0	74.0	92.0	88.0	97.3	100.0
Jan. 26	0.0	2.7	43.3	40.7	66.7	84.0	84.7	72.7	0.0	36.0	52.7	72.0	70.7	84.7	94.7	98.0
Jan. 31	0.0	5.3	39.3	46.0	62.7	79.4	85.4	80.7	0.0	28.7	55.4	58.7	51.3	69.4	97.4	100.0
Feb. 1	0.0	5.3	23.3	52.7	54.7	64.7	68.0	74 0	0.0	25.2	46.7	33 3	43.3	59.4	86.7	98.7
Feb. 3	0.0	2.7	18 0	40.0	54.7	67.4	90.7	88.7	0.0	36.7	44 0	32.7	59.3	67.4	87.4	100.0
% Mortality (based on totals)	0.0	6.00	28.3	42.4	56.9	70.1	81.8	74.7	0.10	32.1	52.1	55.5	65.8	75.7	91.7	99.3

TABLE V

MORTALITIES OF *D. melanogaster* EXPOSED TO DDT BY THE COATED VIAL AND THE IMPREGNATED PAPER TECHNIQUES; TO DETERMINE RELATIVE DIFFERENCES IN THE TWO TECHNIQUES (10 VIALS FOR EACH DOSAGE, REPLICATED EIGHT TIMES, WITH AN EXPOSURE PERIOD OF 24 HR.)

——	Impregnated paper								Coated vial							
Mgm. DDT per 10 ml. acetone	0.00	0.25	0.50	0.75	1.00	1.50	3.00	10.00	0.00	0.25	0.50	0.75	1.00	1.50	3.00	10.00
% Mortality (based on totals)	0.17	2.25	15.0	27.1	42.0	60.6	72.2	51.9	0 50	30.8	55.8	56.8	63.8	70.5	76.7	96.2

TABLE VI

MORTALITIES OF *D. melanogaster* EXPOSED TO TWO BATCHES OF PAPERS SUCCESSIVELY IMPREGNATED WITH THE SAME DDT SOLUTION (2 MGM. OF DDT IN 40 ML. OF ACETONE); TO CHECK POSSIBLE SOLUTION ADSORPTION BY THE SOLUTE (10 VIALS FOR EACH IMPREGNATION, REPLICATED TWICE)

——	Check	First impregnation	Second impregnation
Average percentage mortality	0.33	10.7	11.7

TABLE VII

MORTALITIES OF *D. melanogaster* EXPOSED TO 666 BY THE COATED VIAL AND THE IMPREGNATED PAPER TECHNIQUES; TO DETERMINE RELATIVE DIFFERENCES IN THE TWO TECHNIQUES WHEN AN INSECTICIDE IS USED THAT HAS BOTH A FUMIGANT AND A CONTACT EFFECT (10 VIALS FOR EACH DOSAGE, REPLICATED THREE TIMES)

——	Impregnated paper			Coated vial		
Mgm. 666 per 10 ml. acetone	0.00	0.05	0.10	0.00	0.05	0.10
Average percentage mortality	0.20	95.6	100.0	0.20	52.9	94.5

TABLE VIII*a*

MORTALITIES OF *D. melanogaster* SPRAYED WITH DDD AND WITH DDT (20 VIALS FOR EACH DOSAGE. ENTIRE TEST COMPLETED ON ONE DAY)

——	DDD				DDT			
Mgm. of insecticide per 10 ml. suspension	0.0	1.0	2.0	4.0	0.0	1.0	2.0	4.0
Average percentage mortality	1.67	6.67	14.0	51.3	1.67	37.3	69.3	92.3

TABLE VIII*b*

MORTALITIES OF *D. melanogaster* EXPOSED BY THE COATED VIAL TECHNIQUE TO DDD AND TO DDT. VIALS COATED WITH INSECTICIDE 24 HR. PREVIOUS TO TESTING (40 VIALS FOR EACH DOSAGE, REPLICATED TWICE)

——	DDD						DDT					
Mgm. of insecticide per 10 ml. acetone	0.0	3.0	5.0	8.0	15.0	25.0	0.00	0.20	0.35	0.50	1.00	2.50
Average percentage mortality	0.42	49.8	60.6	69.6	78.8	92.7	0.25	41.7	69.3	76.5	87.4	95.3

TABLE IX

MORTALITIES OF *D. melanogaster* EXPOSED TO THE *o-p* ISOMER OF DDT BY THE IMPREGNATED PAPER TECHNIQUE (10 VIALS FOR EACH DOSAGE, REPLICATED EIGHT TIMES)

Mgm. *o-p* isomer of DDT per 10 ml. alcohol	0.0	50.0	100.0	150.0	200.0	250.0
Date	Average percentage mortality					
Oct. 10	0.6	13.9	42.5	55.6	67.2	70.0
Oct. 11	0.4	14.6	41.4	57.8	69.1	76.6
Oct. 12	0.0	18.2	30.9	45.7	49.3	54.8
Oct. 12	4.7	16.4	36.6	50.3	50.0	48.1
Oct. 14	2.1	14.8	66.0	77.1	88.2	92.1
Oct. 16	1.7	16.6	51.1	47.1	69.5	83.7
Oct. 17	0.6	8.9	20.0	38.3	50.0	51.2
Oct. 18	0.0	6.8	12.2	19.8	38.0	48.4
% Mortality (based on totals)	1.23	13.9	33.0	49.5	60.1	65.7

TABLE X*a*

MORTALITIES OF *D. melanogaster* EXPOSED BY THE IMPREGNATED PAPER TECHNIQUE TO ALL POSSIBLE COMBINATIONS OF FOUR DDT AND FOUR *o-p* DDT DOSAGES; I.E., 16 TREATMENTS IN ALL (10 VIALS PER TREATMENT, REPLICATED EIGHT TIMES)

Mgm. *o-p* DDT per 10 ml. alcohol	Mgm. DDT per 10 ml. of alcohol			
	0 00	0.50	1.00	2.50
	Average percentage mortality			
0 00	1.60	18.5	43.4	79.2
0.25	2 50	18.4	51.7	84.0
1.50	1.75	20.8	44.1	73.3
2 50	0 83	20.0	38.5	72.3

TABLE X*b*

ANALYSIS OF VARIANCE ON MORTALITIES (CONVERTED TO ANGULAR VALUES) OF *D. melanogaster* EXPOSED BY THE IMPREGNATED PAPER TECHNIQUE TO ALL POSSIBLE COMBINATIONS OF FOUR DDT AND FOUR *o-p* DDT DOSAGES

Sources of variation	Sums of squares	Degrees of freedom	Variance	*F* value	*F* value for 5% point
Days	7760.58	7	1108.65	37.16	2.19
DDT dosages	56,641.83	3	18,880.61	—	—
o-p DDT dosages	327.59	3	109.19	3.66	2.70
Interaction *o-p* DDT × DDT	338.52	10	33.85	1.13	2.00
Error	3106.54	104	29.87	—	—
Total	68,175.06	127	—	—	—

Note: For a significant interaction, variance for interaction must be equal to at least 2 × variance for error, i.e., 2 × 29.87 = 59.74.

TABLE XI

MORTALITIES OF *D. melanogaster* EXPOSED BY THE IMPREGNATED PAPER TECHNIQUE TO 1,1-BIS-(*p*-BROMOPHENYL)-2,2,2-TRICHLOROETHANE AND TO DDT (10 VIALS FOR EACH DOSAGE, REPLICATED EIGHT TIMES)

——	1,1-Bis-(*p*-bromophenyl)-2,2,2-trichloroethane								DDT
Mgm. of insecticide per 10 ml. of alcohol	0.0	2.5	5.0	25	35	55	75	100	2.5
% Mortality (based on totals)	1.3	6.35	7.07	13.2	39.2	74.7	88.4	99.7	71.1

TABLE XII*a*

MORTALITIES OF *D. melanogaster* EXPOSED BY THE COATED VIAL TECHNIQUE TO 1,1-BIS-(*p*-FLUOROPHENYL)-2,2,2-TRICHLOROETHANE, AND TO DDT. VIALS COATED ON OCT. 27 (10 VIALS FOR EACH DOSAGE TESTED ON SEVEN SUCCESSIVE DAYS)

——	DDT							1,1-Bis-(*p*-fluorophenyl)-2,2,2-trichloroethane						
Mgm. of insecticide per 10 ml. acetone	0.00	0.10	0.25	0.50	0.75	1.00	1.50	0.00	0.10	0.25	0.50	0.75	1.00	1.50
Date of test	Average percentage mortality													
Oct. 28	0.0	0.7	17.3	48.0	48.0	59.3	65.3	0.7	1.3	0.7	14.0	39.3	62.7	94.0
Oct. 29	0.7	20.0	52.7	60.7	69.3	76.0	82.0	4.0	4.0	6.7	24.7	46.7	82.0	100.0
Oct. 30	6.7	5.3	21.3	52.0	62.7	68.7	82.7	5.3	8.7	2.7	12.0	19.3	23.3	98.0
Oct. 31	1.3	6.7	44.0	66.0	72.7	77.3	82.0	1.3	2.7	1.3	5.3	4.7	6.7	86.7
Nov. 1	4.7	10.0	38.0	48.7	60.0	70.0	74.0	3.3	7.3	7.3	12.7	14.7	17.3	84.7
Nov. 2	2.0	8.7	42.0	61.3	74.7	80.0	84.0	4.0	2.7	1.3	3.3	1.3	2.7	70.0
Nov. 3	0.7	1.3	8.0	30.7	58.7	52.7	55.3	0.7	0.0	0.0	0.7	0.7	1.3	42.7
Nov. 4	0.7	1.3	11.3	43.3	54.7	49.3	67.3	0.7	0.7	0.7	0.0	1.3	0.7	27.3

TABLE XII*b*

MORTALITIES OF *D. melanogaster* EXPOSED BY THE COATED VIAL TECHNIQUE TO 1,1-BIS-(*p*-FLUOROPHENYL)-2,2-2-TRICHLOROETHANE AND TO DDT. VIALS COATED WITH INSECTICIDE 20 HR. PREVIOUS TO TESTING (40 VIALS FOR EACH DOSAGE. ENTIRE TEST COMPLETED ON ONE DAY)

——	1,1-Bis-(*p*-fluorophenyl)-2,2,2-trichloroethane				DDT			
Mgm. of insecticide per 10 ml. of acetone	0.00	0.60	0.85	1.20	0.00	0.60	0.85	1.20
Average percentage mortality	0.50	50.5	81.2	96.8	0.17	49.2	53.5	56.8

TABLE XIII

MORTALITIES OF *D. melanogaster* EXPOSED BY THE IMPREGNATED PAPER TECHNIQUE TO 1,1,BIS-(*p*-METHYLPHENYL)-2,2,2-TRICHLOROETHANE AND TO DDT (10 VIALS FOR EACH DOSAGE, REPLICATED EIGHT TIMES)

—	1,1-Bis-(*p*-methylphenyl)-2,2,2-trichloroethane							DDT						
Mgm. of insecticide per 10 ml. of alcohol	0.0	2.5	5.0	25.0	100	175	500	0.0	0.2	0.5	0.75	1.0	2.5	5.0
% Mortality (based on totals)	0.3	33.6	34.0	34.9	32.1	42.6	65.9	0.6	15.6	32.0	70.3	75.9	92.8	94.1

TABLE XIV

MORTALITIES OF *D. melanogaster* EXPOSED BY THE COATED VIAL TECHNIQUE TO 1-PHENYL-1-*p*-CHLOROPHENYL-2,2,2-TRICHLOROETHANE AND TO DDT (10 VIALS FOR EACH DOSAGE, REPLICATED FOUR TIMES)

—	1-Phenyl-1-*p*-chlorophenyl-2,2,2-trichloroethane						DDT					
Mgm. of insecticide per 10 ml. of acetone	0.00	4.0	12.0	18.0	28.0	40.0	0.00	0.15	0.35	0.50	1.00	2.00
Date of test	Average percentage mortality											
Dec. 12	0.7	22.7	40.0	45.3	64.0	73.4	0.0	18.7	74.7	86.7	94.7	98.0
Dec. 13	0.7	28.7	45.3	48.7	71.4	71.4	0.0	43.3	71.4	95.4	96.0	98.7
Dec. 15	0.0	24.7	38.0	56.7	60.6	76.0	0.0	38.7	74.0	92.7	93.4	94.0
Dec. 17	0.0	5.3	15.3	24.0	46.8	56.0	0.7	15.3	64.0	76.7	92.7	95.4
% Mortality (based on totals)	0.33	20.3	34.7	43.7	60.3	69.2	0.17	29.0	71.0	87.8	94.2	96.5

TABLE XV*a*

COMBINED MORTALITIES OF *D. melanogaster* FROM A SERIES OF EXPERIMENTS INVOLVING 30,000 FLIES EXPOSED TO DDT BY THE IMPREGNATED PAPER TECHNIQUE

Mgm. DDT per 10 ml. alcohol	0.20	0.50	0.75	1.00	2.50
Average percentage mortality	5.95	18.6	41.2	51.1	79.0

TABLE XV*b*

COMBINED MORTALITIES OF *D. melanogaster* FROM TWO EXPERIMENTS INVOLVING 18,000 FLIES EXPOSED TO DDT BY THE COATED VIAL TECHNIQUE

Mgm. DDT per 10 ml. acetone	0.25	0.50	0.75	1.00	1.50	3.00
Average percentage mortality	31.5	54.0	56.2	64.8	73.1	84.2

TABLE XVI*a*

MORTALITIES OF *D. melanogaster* EXPOSED BY THE COATED VIAL TECHNIQUE TO *p*-CHLORO-α-TRICHLOROACETOPHENONE, TO 1,4-CHLOROPHENYL-2,2,2-TRICHLOROETHANOL, AND TO DDT. VIALS COATED ON DEC. 11 (10 VIALS PER DOSAGE FOR EACH TEST. TESTS AT INTERVALS OVER A PERIOD OF SIX DAYS)

—	*p*-Chloro-α-trichloroacetophenone						1,4-Chlorophenyl-2,2,2-trichloroethanol						DDT					
Mgm. of insecticide per 10 ml. acetone	0.0	20.0	25.0	28.0	32.0	40.0	0.00	8.0	12.0	14.0	16.0	20.0	0.0	0.15	0.35	0.50	1.00	2.00
Date of test	Average percentage mortality																	
Dec. 12	0.0	74.0	88.7	99.4	98.7	100.0	0.00	62.7	88.7	92.7	92.7	99.4	0.0	18.7	74.7	86.7	94.7	98.0
Dec. 13	0.0	31.3	63.4	81.4	90.0	100.0	0.67	46.0	76.7	76.0	82.7	91.4	0.0	43.3	71.4	95.4	96.0	98.7
Dec. 15	0.7	2.0	4.0	1.3	2.7	7.3	0.00	12.0	28.0	38.7	42.0	64.0	0.0	38.7	74.0	92.7	93.4	94.0
Dec. 17	0.0	0.7	0.7	0.0	0.0	1.3	0.67	4.0	10.7	26.0	23.3	27.3	0.7	15.3	64.0	76.7	92.7	95.4

TABLE XVI*b*

Mortalities of *D. melanogaster* exposed by the coated vial technique to 1,4-chlorophenyl-2,2,2-trichloroethanol and to DDT. Vials coated with insecticide 12 hr. previous to testing (50 vials for each dosage. Entire test completed on one day)

——	1,4-Chlorophenyl-2,2,2-trichloroethanol						DDT					
Mgm of insecticide per 10 ml acetone	0.00	2.00	4.00	6.00	8.00	10.00	0.00	0.10	0.20	0.30	0.40	0.80
Average percentage mortality	0.27	1.47	5.47	24.5	41.9	83.7	0.40	5.31	24.5	42.0	71.4	92.6

TABLE XVI*c*

Mortalities of *D. melanogaster* exposed by the coated vial technique to *p*-chloro-*α*-trichloroacetophenone and to DDT. Vials coated with insecticide 12 hr. previous to testing (50 vials for each dosage. Entire test completed on one day)

——	*p*-Chloro-*α*-trichloroacetophenone						DDT					
Mgm. of insecticide per 10 ml. acetone	0.00	3.00	8.00	12.00	15.00	20.00	0.00	0.10	0.20	0.30	0.40	0.80
Average percentage mortality	0.40	0.40	34.4	63.6	76.0	87.6	0.53	8.94	38.1	60.9	79.3	95.2

TABLE XVII

Mortalities of *D. melanogaster* exposed by the impregnated paper technique to *p*-chloro-*α*-dichloroacetophenone and to *p*-chloroacetophenone; to determine the residual effect of the two insecticides. Papers impregnated two hours before the date of their first test (10 vials per dosage for each test. Tests at intervals over a period of three months)

——	Acetone check	*p*-Chloro-*α*-dichloro-acetophenone		*p*-Chloroacetophenone	
Mgm. of insecticide per 10 ml. of acetone	0.00	20.0	80.0	20.0	80.0
Date of test	Average percentage mortality				
Jan. 26	1.00	100.0	100.0	100.0	100.0
Jan. 29	0.00	98.0	100.0	98.7	100.0
Apr. 4	0.00	18.7	82.0	10.0	40.0

TABLE XVIII*a*

MORTALITIES OF *D. melanogaster* SPRAYED WITH 666 AND WITH DDT
(20 VIALS FOR EACH DOSAGE, REPLICATED FOUR TIMES)

——	Mgm. of 666 in 10 ml. of 9.5% acetone									Mgm. of DDT in 10 ml. of 9.5% alcohol	
	0.00	0.04	0.05	0.07	0.08	0.09	0.10	0.12	0.15	0.0	1.00
% Mortality (based on totals)	3.14	16.4	27.0	44.4	56.9	65.8	72.4	79.5	89.8	3.32	29.0

TABLE XVIII*b*

MORTALITIES OF *D. melanogaster* EXPOSED BY THE IMPREGNATED PAPER TECHNIQUE TO DDD, TO 666, AND TO DDT; TO COMPARE THE RESIDUAL EFFECTS OF THE THREE INSECTICIDES. PAPERS IMPREGNATED 24 HR. BEFORE THE DATE OF THEIR FIRST TEST (10 VIALS PER DOSAGE FOR EACH TEST. TESTS AT INTERVALS OVER A PERIOD OF FOUR MONTHS)

——	Acetone check	DDD	666			DDT
Mgm. of insecticide per 10 ml. of acetone	0.00	15.00	0.05	0.10	0.25	1.00
Date of test	Average percentage mortality					
Nov. 22/45	0.0	22.7	—	—	100.0	78.7
Nov. 26/45	0.0	8.0	—	—	100.0	62.0
Dec. 1/45	0.0	4.0	—	—	100.0	45.3
Dec. 11/45	1.3	2.7	—	—	100.0	50.0
Jan. 26/46	0.0	0.0	—	—	100.0	56.0
Jan. 31/46	0.0	—	94.7	100.0	100.0	58.0
Mar. 5/46	2.0	—	91.3	100.0	100.0	53.3

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Canadian Journal of Research

Issued by The National Research Council of Canada

VOL. 25, SEC. D. APRIL, 1947 NUMBER 2

A STUDY OF THE WINTER FEEDING HABITS OF THE SHORT-EARED OWL (*Asio flammeus*) IN THE TORONTO REGION[1]

By A. W. F. Banfield[2]

Abstract

The winter feeding habits of the short-eared owl (*Asio flammeus* Pontoppidan) were studied during the period of 1936 to 1942 in the vicinity of Toronto, Ont. The owls used restricted roosts in several groups of Douglas fir (*Pseudotsuga taxifolia*) on a golf course. By means of frequent owl population censuses and collection of pellets, food requirements and correlations were calculated.

The winter incursions of the owls were found to be cyclic and to coincide with the local meadow vole (*Microtus pennsylvanicus*) cycle and to be independent of meteorological factors. Roosting behaviour was found to vary with snow cover.

From an analysis of 3000 pellets it was found that the meadow vole formed 82% of the food taken. The next animal most commonly preyed upon was the deer mouse (*Peromyscus leucopus*), which comprised 17% of the food, while birds formed 1%. No significant seasonal change in diet was noted over a period of five months.

It was calculated that a short-eared owl eats between 700 and 1600 mice per year. The average figure is thought to be nearer the minimum estimate. Translated to weight of mice this is equivalent to between 55.5 and 127 lb.

It was demonstrated that the amount of snow on the ground affected the relative availability of meadow voles and deer mice. This fact was reflected in changes in pressure on the populations of the two mice species due to owl predation.

The data presented pointed to the possibility that concentrations of avian predators could have an appreciable effect on local meadow vole populations. In the case under study it was estimated that winter short-eared owl predation might account for 10% of the vole population.

The data also suggested that during periods of deep snow, because of decreased availability of meadow voles, less food is taken and during open periods increased availability of these mice is reflected by increased food consumption.

Introduction

The short-eared owl (*Asio flammeus* Pontoppidan) has been one of the more favoured birds of prey because of its apparent preference for a diet of meadow voles (*Microtus* sp.). Where the population of these voles had increased to plague proportions, these owls have been noticed to congregate and to feed upon the voles. Such records are given by Adair (1) and Goddard (5) for vole plagues in Scottish border counties.

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[1] *Manuscript received December 30, 1946.*

Contribution from the Department of Zoology, University of Toronto, Toronto, Ont. This paper is based on a thesis submitted as partial requirement for the degree of Master of Arts in the University of Toronto, 1946.

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[The February issue of Section D (Can. J. Research, D, 25 (1) : 1-44. 1947) was issued April 5, 1947.]

At Toronto, during the winter months for the past 10 years, there has been a good opportunity to observe the behaviour of these owls during periods of vole abundance. On Apr. 4, 1936, three short-eared owls were discovered roosting in a clump of Douglas fir (*Pseudotsuga taxifolia*) on York Downs golf course, immediately north of the city limits of Toronto. Each subsequent year the owls have returned in varying numbers to winter at the same locality.

Snyder and Hope (10) recorded the original discovery of the owls, and the analysis of about one thousand pellets that were picked up from beneath the perches. They correlated the appearance of the owls with a generally conceded local peak in the population of meadow voles (*Microtus pennsylvanicus*) during the winter 1935-36.

The following investigation was carried out over a period of seven years with a view to obtaining quantitative data on the food consumption of the owls in the wild state; the effect of certain environmental factors upon feeding habits; and the predator–prey relationship between the owls and the local mouse population.

Throughout the remainder of the paper, the name meadow vole or vole refers to *Microtus pennsylvanicus*, while the term deer mouse refers to either *Peromyscus leucopus noveboracensis* or *Peromyscus maniculatus bairdii*. Both these forms occur at Toronto. *Peromyscus leucopus* is the more common and more general in its habitat choice. *Peromyscus maniculatus bairdii* shows a preference for open wastelands or sandy situations. The great majority of skulls examined from the owl pellets were referable to *Peromyscus leucopus* but it is possible that a few skulls representing the other form were overlooked. The skulls of this genus are usually found crushed in the pellets, which makes identification more difficult.

Location and Habitat

The roosting area used by the owls was of limited extent. They frequented seven clumps of ornamental conifers planted between fairways. Each clump consisted of about a dozen trees, 15 to 20 ft. high, 3 to 6 yd. apart. These clumps were placed about 25 yd. apart along the edges of three fairways. The total area in which these clumps were scattered was about 10 acres. The plantings consisted of Douglas fir (*Pseudotsuga taxifolia*) with a few white cedar (*Thuja occidentalis*) and tamarack (*Larix laricina*) included in the groups. The owls used only the Douglas firs as roosts, probably because they offered denser cover.

Flanking the site on three sides, were deep wooded ravines draining into the Don river valley to the east. On the western side was a waste meadowland of about two hundred acres, dotted with a few cattail marshes surrounded by willows (*Salix* sp.), scattered white elms (*Ulmus americana*), and a few small cottages. This area was separated by a nursery and woodlot from the more extensive uncultivated grassland known as Armour Heights. This whole area was subject only to occasional haying, and controlled spasmodically by local grass fires in spring. It supported an abundant grass and herb vegetation, which proved an admirable habitat for meadow voles.

Method of Study

The area was visited as regularly as possible throughout the residence period of the owls. The site was also visited at different times of day to observe the owls under different daylight conditions. These visits averaged five days apart over the season. All the clumps were approached; the owls were flushed and counted; then observed in flight. The disgorged pellets beneath the roosts were then collected. Pellets are the indigestible remains of a meal, such as fur, feathers, and bones. They are regurgitated in the form of compact balls. Their analysis gives data on the food consumed by the owls. Later in the laboratory these pellets were counted and analysed.

Roosting Behaviour

Early in the study it was noted that when the ground was bare of snow the owls were found roosting on the ground, in the fields. When there was snow on the ground, the owls were found roosting in the Douglas fir clumps on the golf course.

While roosting in the fields, during the day, the owls utilized 'forms'—depressions in the grass clumps. Scattered about were pellets, feathers, fragments of mice, and excreta. These 'forms' were occupied by one or more owls and were usually grouped together. When flushed, several owls rose from a small area. The owls seldom occupied the same forms for two successive nights, although they might roost nearby again.

After a heavy snowfall with the fields and meadowland well covered and only the tips of the weed stalks projecting, the owls were observed roosting in the coniferous clumps on the adjoining golf course. If there were later protracted thaws and the fields again became bare, the owls returned to roosting on the bare ground in the fields.

Under the above described conditions it was found impossible to carry on any quantitative studies while the owls wandered about the fields during the early part of the winter season. But usually by January, under the influence of accumulating snow, the owls would congregate to roost in the conifer clumps during the daylight hours. The collection of quantitative data was then possible. This behaviour will be referred to later in connection with the owl population and illustrated by Figs. 1, 2, 3, 4.

It appeared that this roosting behaviour could be explained as a response to cover requirements. In a bare winter field these brown, mottled owls blended particularly well with the dried grass, and they were usually not seen until flushed from their 'forms'. A heavy fall of snow left the fields with a contrasting white background that was poor cover for the birds. Under these

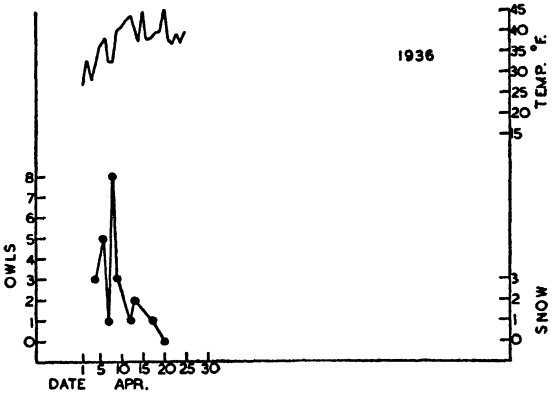

FIG. 1. *Owl population, snowfall, and daily mean temperatures for the winter 1935-6.*

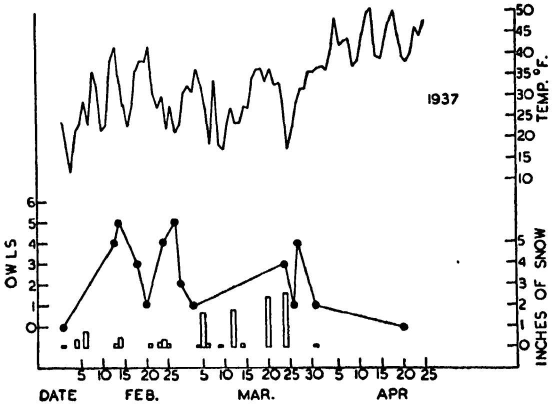

FIG. 2. *Owl population, snowfall, and daily mean temperature for the winter 1936-7.*

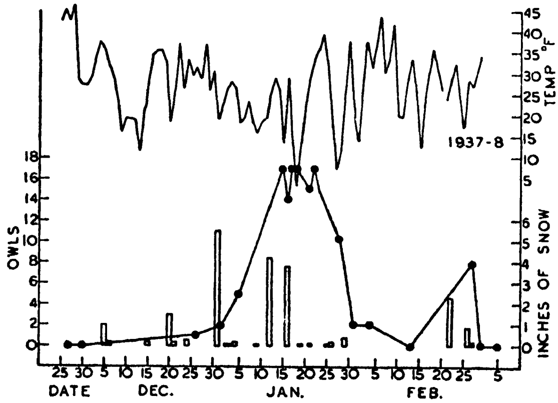

FIG. 3. *Owl population, snowfall, and daily mean temperature for the winter 1937-8.*

conditions the owls roosted in the dense cover of the evergreens. As they perched close to the trunks, they were again unnoticed until flushed.

Predators of the Short-eared Owl

During the winters that the owls were under observation they were comparatively free from molestation by man, since the area they frequented had few houses; the golf course was unused from October until April; and it was unlawful to carry firearms in the area. The above factors tended to give the owls some freedom of movement. During the study no complaints of owl predation on domestic animals were received from the local populace.

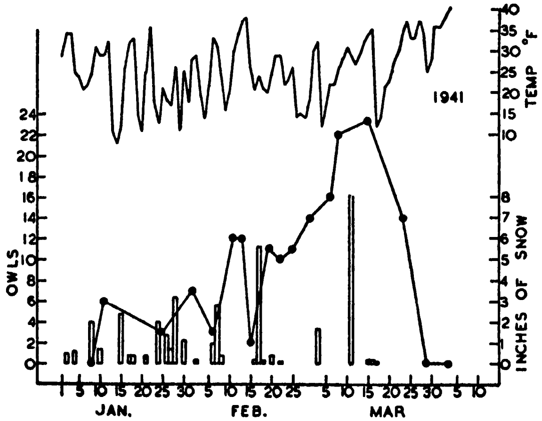

FIG 4. *Owl populations, snowfall, and daily mean temperatures for the winter 1940-1.*

There were present in the area several predaceous animals that might be considered as competitors of the owls, including:

Domestic cat (*Felis* sp.)—a few were seen.

Skunk (*Mephitis mephitis*)—numerous winter tracks and two occupied burrows were found in the owls' hunting range.

Weasel (*Mustela frenata*)—a few tracks were seen.

Marsh hawk (*Circus hudsonicus*)—on two occasions during November and December these hawks were seen to pursue short-eared owls for short distances. On the other hand, Bent (2) gives observations of the short-eared owl pursuing marsh hawks. It is unlikely that these hawks are active predators of the short-eared owl under these conditions.

Great horned owl (*Bubo virginianus*)—observed in the nearby wooded ravines on several occasions.

Goshawk (*Astur atricapillus*)—one was observed flying over the golf course on Dec. 26, 1937.

The only remains of a short-eared owl observed were found in a small ravine on the golf course on Jan. 4, 1938. They gave every indication of a hawk or owl kill, possibly the previously mentioned goshawk.

Yearly Variations in Owl Populations

The owl population during each winter period may be indicated by two methods. The first is by direct observation and calculation of the total number of owl days. One owl day is the total amount of foraging by one owl in a 24 hr. period. The second method of indication is given by the total number of pellets collected from beneath the roosts during the complete winter season. This second method is only roughly indicative of the owl population. There is a secondary significance to this figure, as it also reflects the mouse population.

Returning to the first method, the numbers of short-eared owls flushed from the roosts on the golf course on each observation date are shown on Figs. 1, 2, 3, 4, for the winters 1935-6, 1936-7, 1937-8, and 1940-1, respectively. And on the same figures are shown the mean daily temperatures in ° F. and daily amounts of snowfall in inches. The roosts were not discovered until April, 1936, but there was a previous record of 22 owls 'during the winter'. This explains the great accumulation of pellets collected. During the winters of 1938-39 and 1939-40 the owls did not take up permanent roosts on the golf course but appeared irregularly about the area in comparatively small numbers. Under these latter conditions no quantitative studies were possible.

The peaks in the owl population curves tend to show the local wintering owl population. The dips in the owl curves indicate prolonged mild or snowless periods when the majority of the owls left the conifer roosts and returned to roost in the fields. This phenomenon has been considered previously in the paper.

Fig. 5 shows the total number of owl days for each season and the number of pellets collected from beneath the roosts. Had the study been begun in December, 1935, it is expected that the number of owl days would have reached that of 1940-1. The number of pellets collected shows a marked periodicity with peaks during the winters of 1935-6 and 1940-1.

Fig. 6 shows the number of pellets with two sets of meteorological data, snowfall, and number of day degrees, which are a summation of the differences between the daily mean temperature and 65° F. This gives a measure of the severity of the winter: the greater the total day degrees, the colder the winter.

It is apparent that there is no correlation between these meteorological factors and the winter owl populations. We must look elsewhere for an explanation of their periodic occurrence.

Snyder and Hope (10) presented data to show that locally there had been a peak in numbers of the meadow voles coincident with the influx of short-eared owls during the winter of 1935-6.

During the succeeding winters the numbers of meadow voles decreased locally. And during these winters short-eared owls were present in lesser numbers as indicated on previous figures. No great damage to trees or crops was noted. But during the winter of 1940-1 the population of voles in the uncultivated grassland again assumed plague proportions. A reconnaissance across Armour Heights during the winter showed the fields honeycombed with vole runways and numerous grass nests placed on the average of 3 to 10 yd. apart. Meadow voles were easily caught by hand in haystacks and under debris. On the local roads cars exacted a high mortality on the voles. By spring many small trees and shrubs had been debarked or girdled. During that winter once again there was a heavy influx of owls as indicated by the number of pellets collected during the winter of 1940-41.

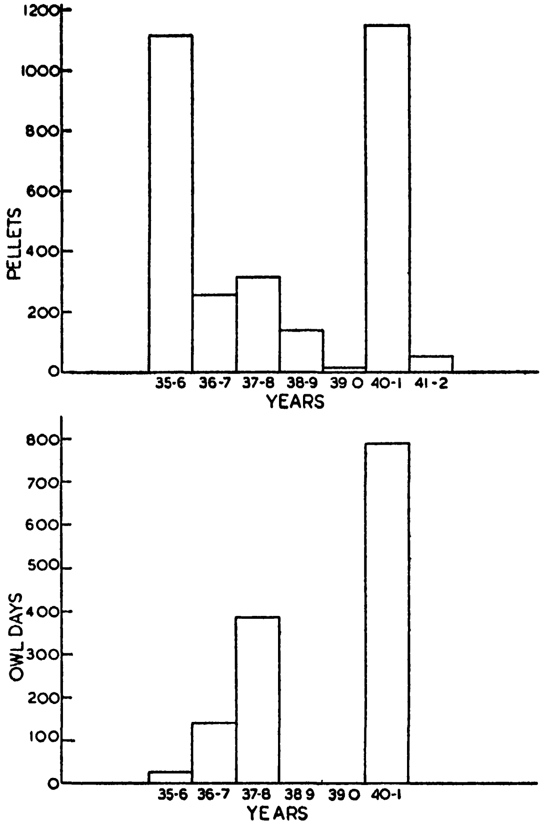

FIG. 5. *The total owl days and the total pellets collected from beneath the roosts for all the winters under study.*

The following winter voles were visibly less numerous and short-eared owls were transitory about the golf course. During the winter of 1945-6 observers stated that voles were abundant in the fields of York Downs and the short-eared owls were present in even greater numbers than before. On Jan. 28, 1946, 36 owls were flushed from the evergreens on the golf course.

Although no quantitative data were collected on vole populations at the locality during the study, it seems safe to assume that locally the vole population showed a marked periodicity with peaks during the winters of 1935-6, 1940-1, and 1945-46. This is based on the observations of several naturalists.

From the present study, with seven winters' data at hand, it appears that the winter incursions of short-eared owls in the Toronto region show a periodicity coincident with the local meadow vole cycle. This, then, is an example of a predator congregating at the locality of its prey's population peak, over a

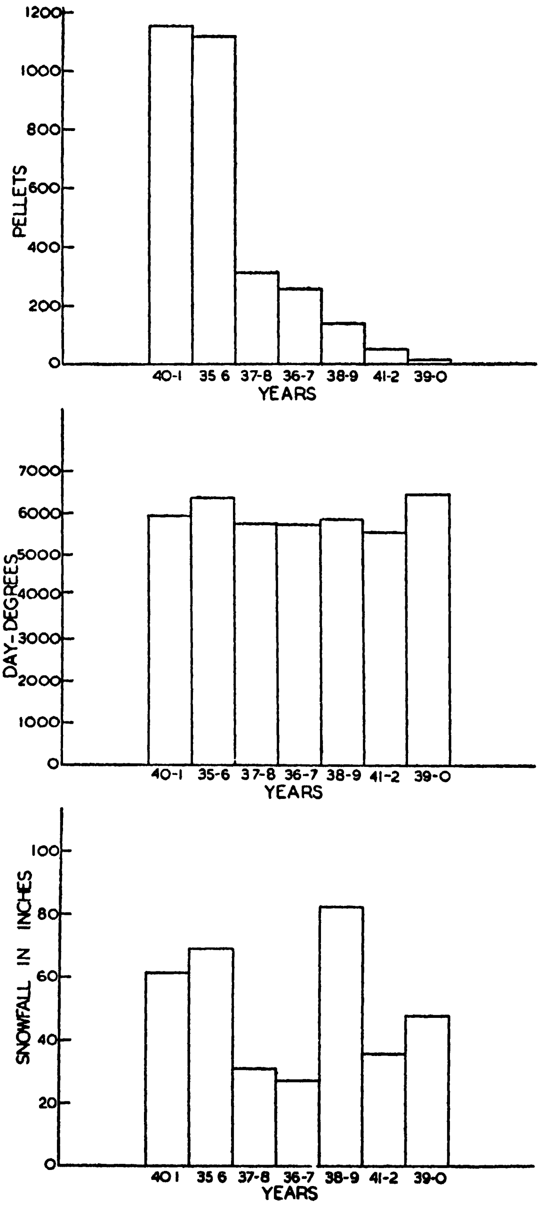

FIG. 6. *Comparison of pellets collected per year, total snowfall, and total day degrees for all years under study.*

period of years; and the predator species therefore showing a local periodicity coincident with its prey's population cycle.

Hamilton (7) believed that there was a regular four-year cycle in the meadow vole populations of New York state He based this conclusion on an exhaustive population study and a collation of population data from other observers. His last reported peak coincided with the local Toronto peak in 1936 But locally at Toronto, from purely qualitative observations, it appeared that the last two vole population peaks showed a five year interval The fact that the area is a more or less uncultivated island may mean that it should not be assumed to be typical

Pellet Analysis

The analysis of the contents of all the pellets collected from beneath the roosts on the golf course for the six years 1935-41, is shown in Table I Results of 1935-6 and 1936-7 are taken from Snyder and Hope (10)

TABLE I

PELLET ANALYSIS BY YEAR

Years		1935 6	1936 7	1937 8	1938 9	1939 40	1940 1	Total
Pellets		1078	252	311	136	12	1145	2934
Animals		1647	415	405	246	19	1666	4398
Microtus	No	1181	389	371	168	18	1475	3602
	%	71 7	93 7	91 8	68 4	94 8	88 6	81 8
Peromyscus	No	450	22	30	73	1	185	761
	%	27 3	5 3	7 5	29 6	5 2	11 1	17 5
Mus	No	1	—		2		1	4
	%	0 1	—	—	0 8	—	0 0	0 1
Plectrophenax	No	8	2	2	2	—	2	16
	%	0 5	0 5	0 5	0 8	–	0 1	0 3
Spizella	No	—	—	—	—	–	3	3
	%	–	—		—	—	0 2	0 1
Otocoris	No	1	1	—	1	–	—	3
	%	0 1	0 2	—	0 4	—	—	0 1
Passer	No	2	—	—		—	—	2
	%	0 1	—	—	–	–	—	0 0
'Birds'	No	4	1	—	1	—	—	6
	%	0 2	0 3	–	0 2	—	—	0 1

It is evident from this table that the meadow vole and deer mouse form the basis of diet for the short-eared owl under these conditions and that all other animals taken are incidental The average percentages of voles and deer mice based on three thousand pellets over six years is 81 8% meadow voles and 17 3% deer mice. For four of the six years the percentage of vole to deer mice remained fairly constantly above this figure. During the winters of 1935-6 and 1938-9, the percentage of deer mice rose to about 30% In this study only the population cycle of the meadow vole has been considered. A cycle in the population of deer mice is quite possible but usually less evident. It is suggested that such a cycle probably affected the availability of deer mice over field voles during these two years. A second factor, which is climatic, will be considered later.

The percentage of birds taken averaged about 0.7% and varied from 0 to 1.2%. This shows that birds are seldom taken under the conditions of the present study. The birds, tree sparrow (*Spizella arborea*), snow bunting (*Plectrophenax nevalis*), horned lark (*Otocoris alpestris*), and house sparrow (*Passer domesticus*) are inhabitants of fields, which are the normal hunting ground of the short-eared owls.

TABLE II

PELLET ANALYSIS BY MONTH, DATA FOR ALL YEARS INCLUDED

Month	Pellets	*Microtus*		*Peromyscus*		*Mus*		Birds		Total
		No.	%	No.	%	No.	%	No.	%	
Nov.	8	7	87.7	0	0.0	1	12.3	0	0.0	8
Dec.	70	61	41.2	82	55.4	0	0.0	5	3.4	148
Jan.	333	387	90.7	37	8.5	0	0.0	3	0.8	427
Feb.	458	642	89.1	66	10.4	0	0.0	3	0.5	721
Mar.	697	978	96.4	33	3.4	1	0.1	1	0.1	1013
Apr.	166	200	93.0	15	7.0	0	0.0	0	0.0	215
Totals	1732	2275	89.9	233	9.4	2	0.2	12	0.5	2532

In Table II the pellets examined are arranged according to the month in which they were collected, in order to show any monthly trends in diet. Voles predominate in every month and their percentage fluctuates around 90% except for December, then there was an evident rise in the percentage of deer mice and a decrease in percentage of voles. Not too much significance should be attached to this, on a monthly diet basis, as the majority of pellets examined for this month came from one lot, which was associated with a period of heavy snowfall. This fact will be considered later.

The percentage of birds taken decreases from December to April. This might reflect the decreasing availability of birds during the late winter when the population is low before the migration influx. But in view of the small percentage under consideration, it appears unlikely that this is of significance. In general it is concluded that there was no appreciable change in diet during the winter months.

Fig. 7 shows the results of the analysis of 1361 pellets collected from 1937 to 1941 based on the number of animals per pellet. It is shown that 55.8% of pellets examined contained one meadow vole and an additional 29.4% contained two meadow voles. Chitty (3) working with a captive short-eared owl has shown that a pellet represents one meal and that it is disgorged before another meal is taken. He also observed that a pellet was held on the average five to seven hours before being ejected. It may be assumed that in the wild state, one pellet represents one forage, because all the contents are in the same state of digestion. From the graph it is noted that in 85% of the forages one or two meadow voles were captured. The average number of deer mice per forage was two or three The deer mouse being smaller than the meadow vole, it takes more of them to satisfy the food requirements of the owls. Finally although birds form an insignificant percentage of the diet, one bird per forage occurred in 60% of the pellets containing birds, suggesting equal food value to the meadow voles.

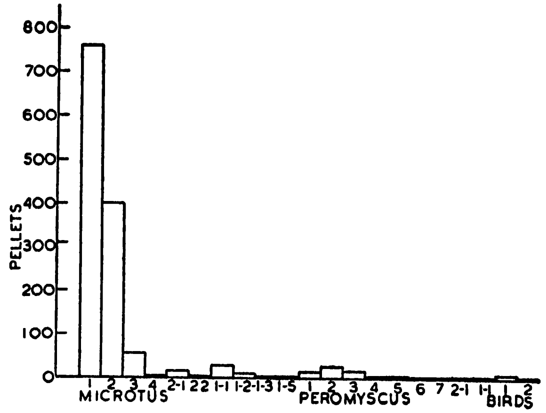

FIG. 7. *The number of animals per pellet.*

From these considerations the maximum food requirements for a short-eared owl may be estimated. From Table I the average number of animals per pellet (forage) is 1.5 approximately. From field observations and the data of Chitty, the number of forages per day would be two to four--at dawn and dusk for certain, possibly at midday and midnight. If we assume an average of three forages per day, in one year 1640 mice would be captured. This is based on optimum considerations and would tend to be a maximum estimate.

Food Consumption Calculations

By counting the squares under the curves, in Figs. 1, 2, 3, 4, the amount of hunting carried out by the owls using the roosts between dates when pellets were collected can be calculated in terms of owl days. By multiplying the number of owl days by the number of pellets collected for the same period, an estimate is reached for the number of pellets disgorged per owl for that period. Finally by multiplying this figure by a figure representing the contents of the pellets examined, an estimate of the food consumed by an owl during that period is arrived at. An analysis of the data for food consumption for the four winters, 1935-6, 1936-7, 1937-8, and 1940-1 is shown in Tables III, IV, V, and VI, respectively. The ratio of pellets to owl days gives a measure of the number of forages. The ratio of skulls to pellets

TABLE III

PELLET AND POPULATION ANALYSIS, 1935-6

Date	Owls	Owl days	Pellets	Pellets	Skulls	Skulls	Skulls	*Microtus*	*Peromyscus*	*Peromyscus*, %
				Owl days		Pellets	Owl days			
Apr. 7	1	0	1078		1647	1.53				27.3
" 8	8									
" 9	3	9.1	18	1.99	24	1.33	2.62	19	5	21.0
" 12	1									
" 13	2	7.5	12	1.60	13	1.08	1.73	12	1	7.5
" 17	1	6.0	1	0.17	3	3.00	0.50	3	0	0.0
" 20	0	1.5	0							
Total		24 5	31		40					
Av.				1.25		1.29	1 66			

TABLE IV

PELLET AND POPULATION ANALYSIS, 1936-7

Date	Owls	Owl days	Pellets	Pellets	Skulls	Skulls	Skulls	*Microtus*	*Peromyscus*	*Mus*	Birds	*Peromyscus*, %
				Owl days		Pellets	Owl days					
Feb. 1	0	0	0									
" 13	4	18	36	2.00	65	1.72	3.47	60	5			8.1
" 14	5											
" 18	3	18.6	132	7.12	219	1 66	12.3	204	13	0	2	5.9
" 21	1											
" 24	4	13.2	3	0.23	6	2.00	0.45	3	2	0	1	33.3
" 27	5											
" 28	2	16.8	9	0.54	17	1.89	1 01	17	0	0	0	0.0
Mar. 3	1	4.4	1	0.23	1	1.00	0 23	1	0	0	0	0.0
" 24	3	43.6	48	1.10	70	1.46	1.61	69	0	0	1	0.0
" 26	1											
" 27	4											
" 31	1											
Apr. 20	0	25.0	23	0.92	30	1.30	1.20	29	1	0	0	3.3
Total		139 6	252		415			380	21		4	
Av.				1.83		1.64	2.99					

remains fairly constant and usually reflects only the size of prey taken. The ratio of skulls to owl days gives a measure of food consumed by an individual owl over the period since the last observation. The percentage of deer mice taken reflects changes in diet as birds form an insignificant part.

From these tables, the number of mice consumed for an arbitrary period can be calculated from the average of skulls per owl days. For convenience the period chosen is a year.

TABLE V

Pellet and population analysis, 1937-8

Date	Owls	Owl days	Pellets	Pellets / Owl days	Skulls	Skulls / Pellets	Skulls / Owl days	*Microtus*	*Pero myscus*	Birds	*Pero myscus*, %
Nov 27	0	0									
“ 30	0										
Dec 26	1	14	7	0 50	9	1 29	0 64	9			0 0
Jan 2	2										
“ 5	5										
“ 15	17	128 4	47	0 36	71	1 51	0 55	61	10		14 0
“ 16	14										
“ 17	17										
“ 18	17										
“ 21	15	95 2	36	0 42	56	1 57	0 59	51	5		9 0
“ 22	17										
“ 23	15										
“ 29	10	110 6	131	1 19	151	1 15	1 37	142	8	1	5 8
“ 30	5										
“ 31	2										
Feb 4	2	19 0	78	4 11	97	1 24	5 11	89	6	2	5 8
“ 12	0	7 2	7	0 97	10	1 43	1 43	10			0 0
“ 27	8	13 4	5	0 40	10	2 00	0 82	9	1		10 0
Mar 1	0		0								
Total		386 8	311		404			371	30	3	
Av				0 81		1 30	1 04				

From 1935-6 data we get 617 animals per year.
1936-7 “ “ “ 1100 “ “ “
1937-8 “ “ “ 382 “ “ “
1940-1 “ “ “ 704 “ “ “

The average based on four years' studies equals 701 animals per year. The study during the winter of 1940-1 was the most critical and its result is considered more reliable It shows close agreement with the average figure of 701 mice per year These calculations show a wide variation represented roughly by 700 ± 45% mice per year (birds are ignored as they form so small a part of the diet) Whether the wide variation in these estimates reflects corresponding differences in the availability of food due to different densities of owls and mice, or are largely due to experimental error cannot at present be determined.

Because of the possibility that all pellets ejected by the owls were not found, these figures give minimum estimates of food consumption. This loss will be considered under discussion It will suffice to conclude here that one short-eared owl would eat at least 700 mice ± 45% based on its winter feeding habits.

To translate this figure to weight of mice, an average weight of 35 7 ± 0 6 gm. for *Microtus* and of 21 9 ± 0 7 gm. for *Peromyscus* has been assumed.

TABLE VI

PELLET AND POPULATION ANALYSIS, 1940-1

Date	Owls	Owl days	Pellets	Pellets / Owl days	Skulls	Skulls / Pellets	Skulls / Owl days	*Microtus*	*Peromyscus*	*Mus*	Birds	*Peromyscus*, %
Jan. 8	0	0	62		138	2.23		51	82	0	5	59.4
" 11	6											
" 25	3											
Feb. 1	7	105.7	41	0.39	52	1.27	0.50	44	8	0	0	15.4
" 6	3	24.6	28	1.14	36	1.29	1.46	33	3	0	0	8.4
" 11	12	35.5	45	1.27	60	1.34	1.70	57	3	0	0	5.0
" 13												
" 15	2	37.5	81	2.15	119	1.47	3.19	82	37	0	0	32.2
" 16	4											
" 19	11											
" 22	10	56.7	50	0.88	65	1.30	1.15	61	4	0	0	6.0
" 25	11	31.5	60	1.91	90	1.50	2.85	83	7	0	0	7.8
Mar. 1	14	50.5	29	0.58	44	1.52	0.88	43	1	0	0	2.0
" 6	16	75.0	95	1.30	141	1.49	1.89	138	3	0	0	2.2
" 8	22	38.0	95	2.50	124	1.30	3.26	121	2	1	0	1.6
" 15	23	157.5	182	1.15	280	1.54	1.78	264	16	0	0	5.5
" 22	14	129.0	150	1.16	200	1.33	1.55	194	6	0	0	3.0
" 29	0	48.0	114	2.38	157	1.38	3.27	152	5	0	0	3.0
" 30	0	0.0										
Apr. 3	0	0.0	10		15	1.50		15	0	0	0	0.0
Total		788.5	970		1521			1338	177	1	5	
Av.				1.23		1.57	1.93					

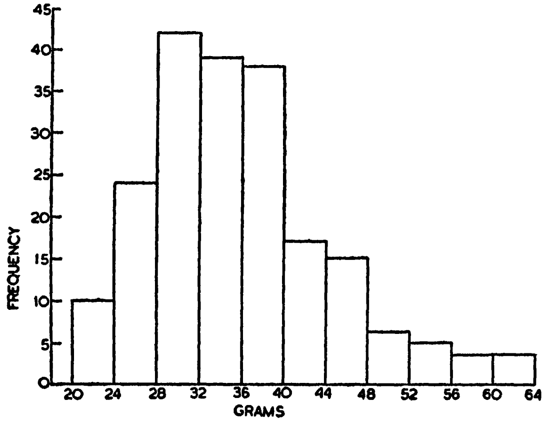

FIG. 8. *Histogram of the weights of 202 adult meadow voles.*

These weights are based on weights of adult Ontario specimens in the Royal Ontario Museum of Zoology. A histogram of the weights of 202 adult *Microtus* is shown in Fig. 8.

Assuming a standard diet of 90% meadow voles and 10% deer mice, it was calculated that a short-eared owl would eat at least 55.5 ± 45% lb. of mice per year, based on the figure of 700 mice per year.

Chitty (3) from feeding experiments on a captive short-eared owl in England concluded that in one year an owl would certainly eat more than 47 lb. of voles, probably more than 95 lb. but less than 142 lb. It is seen that his minimum estimate is close to the above calculation based on feeding under natural conditions. His owl, although mature, gained 12.5% of its original weight during the experiments. Such an increase would be unlikely with winter feeding under natural conditions.

Returning to our estimate of the maximum food requirements of one short-eared owl, of 1640 mice per year, this figure translated to weight gives 127 lb. This figure also shows close agreement with Chitty's data.

He translated this weight to 2000 ± 50% meadow voles per year. He was considering the European meadow vole (*Microtus agrestis*), which would average about 25 gm. in weight as compared to 35.7 gm. for the larger (*Microtus pennsylvanicus*).

Availability of *Peromyscus*

The two chief prey animals of the short-eared owl, the meadow vole and the deer mouse differ widely in their choice of habitat and terrain. The meadow vole is a denizen of meadows and grasslands, where it utilizes a wide ranging system of runways in the grass. It seldom leaves the security of the tunnels and a snowfall gives added protection. The deer mouse, on the other hand, is a forest mouse. It makes its home in thickets, brush piles, fence rows. The deer mouse is a wide ranging mouse whose tracks are commonly seen on the snow.

Hendrickson and Swan (8) working in central Iowa noted that as the temperature went down the percentage of *Peromyscus* in the pellets of short-eared owls increased. Vulnerability of *Microtus* seemed to increase as their tunnels were exposed by melting of the ice and snow. Deep snow serves as a protection for voles and puts the pressure on deer mice.

This relationship has been looked for in the present study. Fig. 9 shows the percentage of deer mice in the pellets plotted with the snowfall in inches and the daily mean temperature for the observations of 1937-8. The percentage of deer mice is plotted as a series of straight lines since this percentage refers to a group of pellets collected between two collecting dates. The snowfall in inches of snow is plotted on a daily basis during the period of observation: as is also the daily mean temperatures.

From a casual inspection of the graph it appears that there is a rough correlation between the percentage of deer mice and the snowfall amount—the heavier the snowfall the higher the percentage of deer mice in the pellets. The relationship is not strict because of the high experimental error. There was a strong possibility of pellets being buried in the snow and not retrieved until later. Actually we are interested in the snow on the ground rather than the snowfall. The relationship between snowfall and lying snow is a complex one. Such factors as daily temperatures, cloudiness, evaporation, must be considered, so that a rigid correlation analysis is beyond the scope of this study. A test of significance of the correlation between snowfall and percentage of deer mice would give a measure of the significance that snowfall plays in the availability of voles and deer mice.

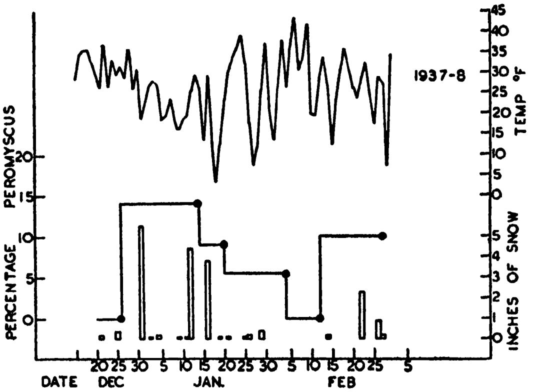

Fig 9 *Comparison of percentage deer mice, snowfall, and daily mean temperature for 1937-8 records*

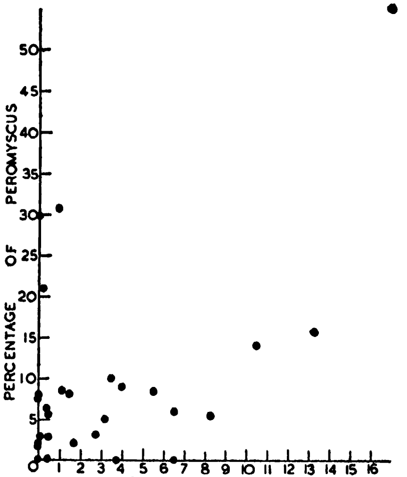

Fig 10 *Percentage of deer mice plotted against snowfall—total data included* $r = +0\ 625$, $t = 5\ 52$, $P < 001$

Such a test of significance of the correlation was carried out. Fig. 10 shows a plotting of percentage of deer mice against snowfall for the period to which the pellets refer The graph contains the complete data from the four winter's data presented in Figs. 1, 2, 3, 4, 5, 6.

There is a rough correlation apparent between snowfall and the percentage of deer mice. Standard statistical procedures were applied to these data. The coefficient of correlation between the snowfall and the percentage of deer mice in the pellets was found to be 0.625. The significance of this coefficient was tested by means of 'Students' t test; t was calculated to be 5.521. From Fisher's tables it was found that one would not expect a coefficient of correlation of such a magnitude in more than one out of 1000 chances from a random uncorrelated population. So we conclude that there is significant correlation between percentage of deer mice in the pellets and the amount of snowfall during the period. No significant correlation between percentage of deer mice in the pellets and average daily mean temperature was found.

We may conclude that snowfall affects the availability of meadow voles and deer mice. During periods of heavy snow meadow voles are protected by a blanket of snow. The pressure of predation by short-eared owls is then transferred to the deer mice, which are more available to the owl

Animals Eaten per Day

It is the generally held opinion, based on physiological requirements, that, in order to maintain their heat requirements, active homoiothermic animals must eat more during cold weather than warm weather. This concept does not take into account the normal environmental condition in which an animal is usually situated.

In Canada periods of low temperature are usually associated with conditions of snow, frozen ponds, perhaps hard packed snow. To such an animal as the short-eared owl, these periods are times when natural food in the form of meadow voles is less available. Under these conditions it is unlikely that the owls are able to eat more to maintain their heat requirements. It is more likely that they have to eat heavily when food is plentiful during mild snowless periods and tide themselves over periods with cold frozen snow covered landscapes. It is during these periods of deep snow and low temperatures that one often finds animals dead from starvation or exposure.

From our general analysis Tables II, III, IV, V, we have a series of figures giving the skulls per owl day. These figures give a measure of the average number of animals captured by the owls over the previous period. From inspection this figure is found to vary widely, changing climatic factors would be one plausible reason why the owls ate more during certain periods than others. Fig. 11 shows the average number of animals eaten per day for the pellet period, plotted for the season, 1940-1, and plotted against this figure are the amount of snowfall and daily mean temperatures.

A rough correlation between animals per day and the climatic factors is evident. The greatest number of animals eaten per day occur when the weather has been mild and the snow melting, or the ground bare. The lower numbers of animals eaten were associated with cold spells and deep snow, the relationship of all the factors is exceedingly complex, and its analysis difficult.

A correlation between animals per day and snowfall would support the hypothesis that hunting was more successful with the vole runways exposed by melting snows and mild temperatures. During these periods the owls were able to feed heavily and tide themselves over the periods when foraging was difficult in cold spells or heavy snowfalls.

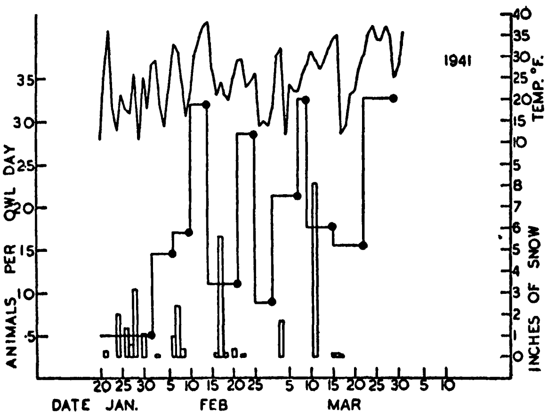

FIG. 11. *Number of animals eaten per day plotted against snowfall for period, 1940-1 date.*

Fig. 12 shows a plotting of animals per day against snowfall for all the observations over all the years studied. There is a suggestion of correlation between animals per day and snowfall. A calculation of the coefficient of correlation between animals per day and snowfall was found to give an r equal to $-.2041$. The negative coefficient signifies that a rise in the amount

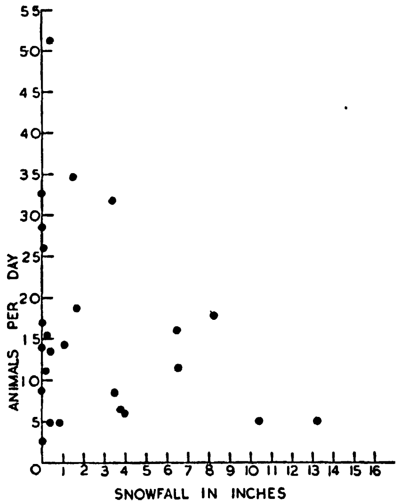

FIG. 12. *Animals eaten per day plotted against snowfall during period—total data.* $r = -0.2041$; $t = 1.8$; $P = <0.1$.

is accompanied by a decrease in animals per pellet. When this '*r*' was tested by means of 'Students' *t*, it was found that an *r* of this magnitude would be expected in about one out of 15 random samples from an uncorrelated population. As this is about the rejection point, this simple correlation between snowfall and animals per day is not considered significant, and the test has failed to prove conclusively our thesis. One of the factors that obscures the relationship is the fact that the owls consume more deer mice than meadow voles per forage. During periods of snowfall deer mice are more available than voles. These two factors would combine to give more skulls per day during periods of heavy snowfall, and thus obscure the correlation under consideration.

Although the test of correlation fails to support the hypothesis with certainty it is still thought worthy of consideration.

Predation Effect

The territory over which the owls hunted was carefully observed during daylight hours and at dusk. It appeared restricted to the uncultivated meadowland to the west of the golf course This area was estimated by rough measurement to be approximately 200 acres. During the winter of 1940-1, the owls were present at York Downs in considerable numbers from Nov. 15 to Apr. 15. Referring to graph Number 4 it seems reasonable to assume a local population of 20 owls During that same winter period their predation on meadow voles was at least,

$$700 \times \frac{5}{12} \times 20 \times \frac{9}{10} = 5250 \text{ voles.}$$

From this study no figures on meadow vole populations were obtained, but Hamilton (7) gave a vole population of 50 to 250 per acre (the latter figure for peak years). Assuming 250 voles per acre, a population of 50,000 meadow voles was estimated on the hunting grounds The short-eared owls' predation would then account for at least 10.5% of the population. These figures are conservative estimates based on a minimum food requirements estimate. They suggest that the predation of these owls would have an appreciable effect on the vole population.

The short-eared owl was only one of several active predators that preyed on the voles. Others present were snowy owl (*Nyctea nyctea*), horned owl (*Bubo virginianus*), red-tailed hawk (*Buteo borelis*), and American rough-legged hawk (*Buteo lagopus*). These avian predators alone could conceivably exact up to 20% mortality on the vole population, and occurring at a time when reproduction was at a minimum, this predation might be an important factor in controlling the local vole population.

Elton (4) has shown from the works of Russian ecologists that they favour the theory that predators exercise control on rodent population peaks brought about by optimum food conditions. This theory is expounded by Kalabukhov (9) and is supported by an accumulation of ecological studies along lines similar to the present study. There have been population studies on voles by means of metal tagging. These tags have been retrieved from the pellets of predaceous birds. There are also studies on pellet analysis from standard perches. All these studies suggest that the role of predators in controlling rodent populations may be greater than is usually accepted by American ecologists.

Discussion

Naturally these calculations are subject to considerable error. The chief source of error arises from the possibility that all the pellets disgorged by the owls during the period were not collected. With reference to this possibility it may be said that the roosts were so restricted that probably all the pellets disgorged by the owls at the roosts were collected. One must, however, consider the possibility of other roosts.

Guerin (6), working with the barn owl (*Tyto alba*) in France, established the fact that there were at least two daily ejections of pellets—"pelote nocturne and pelote diurne". The former followed a meal taken perhaps within three minutes from the beginning of the night's hunting. It was produced at the "station nocturne" before the owl took its second meal (about 5 a.m.) in July and returned to roost at the "station diurne" where the second pellet was produced. No similar behaviour has been described for the short-eared owl. According to Bent (2) short-eared owls hunt at dawn, dusk, and spasmodically throughout the day. In the present study, any pellets disgorged from the dawn forage would be at the "station diurne"—in this case the ornamental fir clumps. The pellets from any diurnal forages would also be deposited here. Unless the owls returned to roost at night in the evergreens the pellets from the dusk forage would be dropped elsewhere.

The hunting grounds were intensively covered, and no evidence of a "station nocturne" was found. A quantitative analysis of food consumption based on pellets collected from the "station diurne" tends to give a minimum estimate. At present it is impossible to assess the loss in pellets from the dusk forage. It could theoretically amount to 50%, if there were two pellet stations.

A second source of error from the possibility of shifting owl population between observations must be considered. These shifts were equally probable in either direction and therefore would have a cancelling effect.

There is one consideration that favours this method of study. It is that observation on large groups of animals over long periods of time, and under natural conditions would tend to give more natural results than experimentation on individuals in captivity.

Acknowledgments

I wish to express my appreciation of the assistance and advice given during this study by Prof. J. R. Dymond, Prof. A. F. Coventry, and Dr. J. MacArthur of the Department of Zoology of the University of Toronto.

I am indebted to the staff of the Royal Ontario Museum of Zoology for the use of the museum files and collections. I wish to acknowledge the friendly co-operation of Mr. C. E. Hope who examined about 50% of the pellets analysed in the study, including the data previously reported by Snyder and Hope (10). Mr. J. L. Baillie was of assistance in bringing to notice observations of other naturalists. Mr. S. C. Downing made available the mammal collection file for a compilation of weights.

Meteorological data were supplied by the Toronto Meteorological Office, Department of Transport.

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SOME EFFECTS OF TEMPERATURE ON THE PRODUCTION AND ELIMINATION OF DIAPAUSE IN THE WHEAT STEM SAWFLY, *CEPHUS CINCTUS* NORT.[1]

By R. W. Salt[2]

Abstract

Fully grown *Cephus cinctus* larvae go into an obligatory diapause, the ending of which involves the elimination of two separate factors. The first, or x factor, is eliminated only at subdevelopmental temperatures and has a positive temperature coefficient in a temperature range from the undercooling point up to the developmental threshold. The second, or y factor, is eliminated at either low or moderate temperatures, having a positive coefficient in a temperature range from the undercooling point up to about 30° C. When the x factor reaction is complete, diapause may be said to be 'broken', but it is not 'eliminated' until the y factor reaction is complete.

When the x factor reaction is complete but the y factor is still present, the insect can be returned to diapause by either high temperatures or a lack of adequate moisture or a combination of these. This sometimes occurs in nature, resulting in a two-year life cycle. Once the y factor has been eliminated the insect starts postdiapause development and can no longer return to a state of diapause.

Postdiapause development under constant favourable conditions is rather uniform after it has once started, but the time of its initiation is variable, dependent on the y factor. Evidence is presented that indicates that the y factor is eliminated faster in large larvae than in smaller ones, accounting for the rather wide variability in development in samples uniformly treated but not selected as to size.

At 10° C. roughly 40 to 110 days were required to break diapause, each larva requiring a definite conditioning period. Thus the process ends abruptly in individuals, gradually in a group. Under natural conditions diapause was broken as early as Oct. 19, 1945, in a few cases, and was broken in all cases by the end of January 1946.

Introduction

The wheat stem sawfly, *Cephus cinctus* Nort., is a univoltine species with an obligatory diapause in the mature larval stage. It reaches this stage in the late summer, and the diapause, which prevents further development in the fall when food and temperature conditions are unfavourable, is eliminated by the time spring temperatures rise sufficiently to allow development. The occurrence and elimination of diapause during the fall and winter is so universal that it would be of mere academic interest were it not for the fact that diapause can be reinstated in the spring. Farstad (2) has found that this "spring diapause" may affect large percentages of the larval population under drought conditions or under similar conditions created in stubble fields where shallow tillage operations have raised the infested stubs* to the soil surface. Small

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[1] *Manuscript received November 22, 1946.*

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**The term stub refers to the short stubble that remains after the larva cuts the grain stem, about the time the grain is ripening. The stem is weakened at the cut, made just above the soil surface, and readily breaks off. The larva plugs the end of the stub with frass and lines the entire cavity with a cocoon. It spends the fall, winter, and spring in the stub, changing to pupa and adult before emerging through the frass plug in June.*

numbers of larvae in spring diapause are commonly found throughout western Canada as a result of localized drought conditions in their microclimates. Soil erosion by wind or water, whether on a large or small scale, tends to expose stubs to subsequent heat and desiccation. The chances of larvae in such stubs re-entering diapause are much greater than those in stubs with the normal protection of the surrounding soil. Thus a study of diapause in *Cephus cinctus* is of practical importance.

The literature on diapause in insects is so voluminous that no review of it will be attempted here. Reviews are contained in other papers, a recent one being given by Prebble (3-7). The phenomenon of diapause is so widespread among insects that its manifestations are variable. It may occur in univoltine, bivoltine, or multivoltine species. It may occur in feeding or non-feeding stages, the latter being most common. It may be of long or short duration, and it may be produced and destroyed by a variety of factors.

Diapause in *Cephus Cinctus*

In *Cephus cinctus* diapause occurs only in the fully grown larva in the stub. By this time the larva has completed feeding, emptied the gut, and lined the stub with a cocoon. It has a characteristic S-shape, which persists until after diapause has been eliminated and development is resumed, when it straightens out to become a prepupa. No ecdysis accompanies this change, but as the larva becomes straight, constrictions appear between the head and thorax and between the thorax and abdomen, producing three distinct body regions. In this paper the two stages will be referred to as S-larva and prepupa, but it will be seen later that while they are morphologically distinct, important physiological changes occur that do not coincide exactly with the externally apparent morphological change. It is impossible, for example, to tell at a glance whether an S-larva is in or out of diapause. Under both conditions they wriggle actively, and it is only by the use of some physiological test that the distinction can be made, unless one waits until the prepupal stage is either reached (diapause broken) or fails to be reached (diapause intact). This latter method, however, is rather unsatisfactory, for it is not only time-consuming (several weeks may be required) but it also introduces the disadvantages of uncertain rearing techniques. For while, if an S-larva becomes prepupal at ordinary rearing conditions, it is certain that its diapause had been broken, on the other hand if it fails to become prepupal it is not certain that its diapause was not broken. It will be shown later that a larva in which diapause is broken may return to a state of diapause under certain adverse conditions. In experimental work such conditions were often difficult to avoid when it was necessary to remove the larvae from their stubs. Rearing methods are therefore of considerable importance.

Under natural field conditions diapause in *Cephus cinctus* is obligatory in that all larvae without exception are in diapause when they cut the stem in the fall. Similarly diapause is broken during the fall and winter in all individuals. The finding of diapause larvae in the spring and summer indicates a return to diapause and not a failure of winter conditions to break diapause.

Materials and Methods

Infested stubs were either collected fresh from wheat stubble when required or taken from large collections made in the fall and stored for later use under a variety of conditions. Since the storage conditions are of great importance they are specified later for each experiment as it is described.

All stubs were collected from fields of Marquis or Red Bobs wheat, both highly susceptible varieties. Constant temperatures were maintained with reasonable accuracy in thermostatically controlled cabinets. Temperatures above room temperature were maintained within a range of about 2° C., while refrigerated cabinets varied within 4° or 5° C. The extremes of variation, however, were infrequent, and occupied only a small percentage of the time involved. If it had been possible to record them, the mean temperatures would undoubtedly have been close to the values listed, and the standard deviations less than 0 5° C.

Relative humidity was controlled by the use of sulphuric acid and water mixtures in glass containers. The air in these was not circulated and was disturbed as little as possible during its period of use.

Adequate moisture is required for development following the breaking of diapause. This applies particularly to the early stages of postdiapause development. Tolerance of moist conditions at this time appears to be quite wide, but dry conditions readily prevent the initiation of development. Unless otherwise stated, stubs used in this work were kept at all times in damp sifted loam with a moisture content (dry weight basis) of 14 to 17%. Early in these studies this method was found to be very satisfactory for both storage and conditioning of infested stubs. At low temperatures storage can be maintained for many months without harm; at higher temperatures the fibrous structure of the stubs breaks down after about three months, and many larvae mould and die.

In some of the work it was necessary to remove the larvae from their stubs and cocoons and retain them for observation. As naked specimens are an easy prey to desiccation on the one hand and moulds on the other, it was difficult to develop a satisfactory rearing technique. Dryness was the lesser of the two evils, as it merely inhibited development, whereas excessive moisture resulted in a high mortality. A fairly suitable method, and one that was of necessity adopted, was to place the specimens in 1 in. lengths of 1 5 to 2.0 mm. (inside diameter) glass tubing plugged at each end with cotton batting. The small air space was presumably kept fairly moist by evaporation from the specimen, thus producing a reasonably satisfactory microclimate. This technique worked fairly well if the specimen had already begun developing, but not otherwise. Whenever the experiment allowed, the insects were left in the stubs in moist soil as long as possible, usually for 20 days at 25° C.

Samples of stubs, when split open, often yielded specimens in various stages of development. A mere list of the number or percentage of each stage or substage is not only unwieldy but it fails to give a quick means of judging the average extent of development. To partially remedy this situation a numerical rating was given to each developmental category, from which a single value, the developmental index, was derived. The range of development within a sample is not indicated, but the average is given at a glance. Since we are accustomed to thinking in terms of percentage, the index has a base of 100 so that it can be thought of in terms of average percentage of development. The factors were chosen on the basis of the approximate duration of each stage, since development from prepupa to adult at constant favourable conditions is a function of time. Since the time intervals from prepupa to early (uncoloured) pupa, early pupa to late (coloured) pupa, and late pupa to adult are approximately equal, and each is about half as long as that between non-diapause S-larva and prepupa, the following factors are suitable and are used in this work.

S-larva	0
Prepupa	2
Early pupa	3
Late pupa	4
Adult	5

The S-larva, being a non-developing form, naturally receives a zero rating. The percentage of each category present in a sample is multiplied by its factor, and the sum of the products is divided by five, giving a developmental index ranging from 0 to 100. In experimental work where only the developing forms are to be considered the S-larvae may be excluded from the samples and the percentage based on developing forms only ($D\ I./x$, where x equals developing forms only, rather than $D\ I\ /s$, where s equals the total sample). For example, if a sample of 40 specimens contains 20 S-larvae, 10 prepupae, 6 early pupae, 2 late pupae, and 2 adults, the developmental index of the complete sample ($D.I\ /s$) will be 28, computed as follows:

$$\frac{50(0) + 25(2) + 15(3) + 5(4) + 5(5)}{5}.$$

The inclusion of the S-larvae presupposes that they are postdiapause and will shortly develop into prepupae If, however, it is known that they are in diapause and will therefore not develop with the remainder of the sample without special conditioning, they should be omitted in the calculations, and the developmental index ($D.I./x$) calculated as follows:

$$\frac{50(2) + 30(3) + 10(4) + 10(5)}{5}.$$

In this case the index is 56. The choice of index therefore depends on whether the S-larvae are in or out of diapause. If this is not known the specimens should be given an opportunity to develop further and the $D\ I\ /x$ index used, where x equals the number of specimens ultimately showing development, i.e. reaching at least the prepupal stage

In all of the work included in this paper the prepupa was distinguishable from the S-larva by appearance only. It is of course obvious that after diapause has been eliminated, the change from S-larva to prepupa requires time. Since the process has a starting point, the insect strictly speaking is a prepupa from that time on, rather than from the somewhat later time at which it becomes visually distinguishable as a prepupa. The intervening period is of great practical importance, for, as will be shown later, a post-diapause S-larva can be returned to a state of diapause before the change to prepupa begins, but not after it begins, even though it still has the appearance of an S-larva. Physiologically, the latter is a prepupa; morphologically, it is an S-larva. To avoid confusion it will be termed an S-prepupa.

Experimental Results

1. *Effects of High Temperatures on Postdiapause Development*

In a previous study dealing with the desiccation of *Cephus cinctus* larvae (8), it had become apparent that high temperatures (35°, 40° C.) adversely affected the development of postdiapause larvae, either preventing development entirely when the exposure was made on early postdiapause larvae, or else resulting in abnormalities, coma, and death when more developed forms were used. In a preliminary test 60 isolated naked larvae that were definitely postdiapause were exposed to a temperature of 35° C., half at 50% and half at 80% relative humidity. Observations were made frequently over a period of three weeks, during which no visible change occurred in the 50% relative humidity group and three became prepupal in the 80% relative humidity group. These three were not normal, and it seems probable that they were so close to becoming prepupal at the start of the experiment that development proceeded in spite of adverse conditions that made such development abnormal.

From this it was established only that the temperature and humidity conditions used interfered with normal development of naked postdiapause larvae. In order to remove the moisture factor as a variable and also any possible effects due to removal from the stubs and cocoons, subsequent exposures to high temperatures were made on larvae in their stubs, in damp soil that provided optimum or near-optimum moisture conditions. Three series were exposed to 35° C., each differing in pre-experimental treatment, as follows. In Series *A*, started Jan. 12, 1945, the stubs had been stored in a root cellar during the preceding 113 days; in Series *B*, started Mar. 14, 1945, the stubs had been stored in a root cellar during the preceding 174 days; in Series *C*, started Mar. 24, 1945, the stubs were collected from a wheat field two days earlier. At the end of successive five-day intervals, from 5 to 40 days, stubs were removed and split until 20 specimens were obtained from each series. No development occurred in any sample of any series. Development, therefore, was either prohibited or else the larvae were returned to diapause. The alternatives were tested, in Series *B* and *C*, by removing duplicate samples of stubs from 35° C. at each five-day interval from 5 to 30 days. Instead of being split at once, these samples were incubated at 25° C., still in moist soil, for a further 20 days, at which time the stubs were split and development recorded. Table I lists the percentage that showed visible development after such treatment.

TABLE I

PERCENTAGE OF DEVELOPING SPECIMENS AFTER 5 TO 30 DAYS AT 35° C., FOLLOWED BY 20 DAYS AT 25° C.

No. of days at 35° C.	Series *B*	Series *C*
5	95	100
10	10	60
15	0	20
20	5	5
25	0	0
30	0	0

It is seen that postdiapause larvae that were exposed to 35° C. for a sufficiently long period (25 days or more) were unable to develop after being returned to favourable conditions, or in other words, diapause was reinstated. If the period of exposure was reduced below 25 days, the proportion of developing specimens increased; virtually all specimens developed after only five days at 35° C. Thus a very definite amount of time is necessary to put a larva back into diapause, though this time varies among larvae. Of the two series above, Series *B* was the more advanced. Less time was required to produce diapause in it than in Series *C*, indicating that each larva must reach a certain point or condition before the high temperature produces its effect.

The objection might be raised that 20 days' incubation at 25° C. was insufficient to allow recovery from the high temperature in the event that there were any effects other than the reinstatement of diapause. For this reason Series *C* was duplicated on May 29, 1945, using stubs from the same collection as before, but that had been stored in damp soil at 2° C. during the intervening 68 days. In addition, an extra supply of these stubs was exposed to 35° C. for 25 days. These were then placed at 25° C. and sampled at 10, 15, 20, 25, 30, 40, 50, and 60 days thereafter. The results closely paralleled those of Series *C* in Table I In the extra 25-day samples no development occurred even after incubation at 25° C. for as much as 60 days. The conclusion that the larvae were actually returned to a lasting diapause seems amply justified.

Provided that moisture conditions are suitable, a temperature of 35° C. has no deleterious effects on diapause larvae (8), while in non-diapause larvae, diapause is reinstated. Its effects on prepupae and pupae are quite different, resulting in comatose and abnormal forms. Postdiapause larvae were allowed to develop in their stubs at 25° C. for various periods, after which they were held at 35° C. for periods of 10 and 20 days. The results are presented in Table II.

TABLE II

EFFECTS OF A TEMPERATURE OF 35° C. ON *Cephus cinctus* AFTER POSTDIAPAUSE DEVELOPMENT HAS STARTED

Days at 25° C.	Days at 35° C.	Percentage				Percentage of developing forms
		S-larvae	Prepupae	Early pupae	Late pupae	
5	10	100	0	0	0	0
5	20	100	0	0	0	0
10	10	40	60	0	0	60
10	20	50	50	0	0	50
15	10	0	80	20	0	100
15	20	7	62	31	0	93
20	10	5	50	5	40	95
20	20	18	29	6	47	82
25	10	0	0	5	95	100
25	20	0	5	0	95	100

Virtually all of the *developing* specimens were comatose or dead, the latter being either moulded or flaccid. It was obvious throughout the course of the experiment that the higher temperature had halted development at whatever point had been reached at 25° C. In other words, development was prohibited at 35° C. There were no apparent ill effects on the non-developing S-larvae but prepupae and pupae lapsed into a coma from which they did not recover. Abnormalities appeared in the prepupae, many of which slowly acquired a semipupal appearance without moulting. First the head darkened and became shaped somewhat like a pupal head; then the thorax gradually darkened, to resemble the form and coloration of the late pupa, but without legs or wings. No further major morphological changes occurred, and the specimens gradually died, sometimes after surviving three or four weeks

2. *Effects of Size on Postdiapause Development*

There is considerable variation in size among mature *Cephus cinctus* larvae, attributable mainly to differences in the nutrition of the feeding larvae, which in turn is dependent largely on the growth and vigour of the host plant (2). It became rather noticeable in the course of rearing larvae through to the prepupal, pupal, and adult stages that the larger specimens usually developed sooner. This is not to say that the larger ones developed at a faster rate than the smaller ones, but that they started developing sooner. The speed-up occurs early in the postdiapause period, certainly before the appearance of the prepupa. At present the details are lacking, but a possible explanation of the phenomenon is discussed later.

A sample of 92 specimens, incubated at 25° C. for 20 days in soil of 14% moisture content was divided into groups on the basis of development, weighed, and the moisture and dry matter contents determined by oven-drying to constant weight at 95° to 100° C. These data are listed in Table III.

TABLE III

COMPARATIVE WEIGHTS OF DEVELOPING AND NON-DEVELOPING STAGES IN A SAMPLE OF INCUBATED POSTDIAPAUSE *Cephus cinctus*

——	Number	Average weight, mgm.	Average dry matter, mgm	Average moisture content, %
S-larvae	15	4.2	1.6	61.5
Prepupae	17	5.0	1.8	63.0
Early pupae	60	8.5	3.2	62.3

There is no significant difference in moisture or dry matter contents, although these differed in absolute amounts. The more advanced forms were definitely larger and heavier than the less advanced.

This point was checked again in the course of another experiment in which each pupa was weighed as soon as it was formed. Table IV lists the number of days required by these specimens to pupate at 25° C.

TABLE IV

RELATION BETWEEN SIZE AND NUMBER OF DAYS TO PUPATION

No. of days to pupation at 25° C	No. of specimens	Average weight, mgm
20-21	25	10.1
22	17	8.2
23	14	5.9
24	5	6.7
25	3	5.7
26-28	17	5.4

Again it is evident that in general the larger specimens are more advanced in a random sample of uniformly treated stubs.

3. *Effects of Moderate Temperatures on Diapause in Cephus cinctus*

Not a single case has been recorded of the elimination of diapause at moderate temperatures (20° to 30° C.). Thousands of infested stubs have been stored at room temperature and at constant temperatures within this range, under a variety of moisture conditions, for periods of up to 16 months. Not only is diapause not broken at such temperatures, but there is no indication of any effect whatsoever on it. It is therefore concluded that diapause in *Cephus cinctus* is not only obligatory but is also maintained at moderate and high temperatures.

4. *Effects of Low Temperatures on Diapause in Cephus cinctus*

The diapause present in every *Cephus cinctus* larva in the fall is broken by spring. It was at first largely taken for granted that low temperatures were responsible for the change. If postdiapause material was needed for winter experimental work, fall-collected stubs were placed on or near the coils of a refrigerator or in a root cellar for the greater part of the winter. This procedure was quite successful when sufficient conditioning was allowed. No precise information was at hand, however, to indicate what temperature conditions were most suitable, or whether the time factor could be reduced. In order to get such information, attempts were made in the fall of 1944 to break diapause under controlled conditions.

The first exposures were made on Sept. 8, 1944, using infested stubs collected the previous day. The temperature of the refrigerator varied between 2° and 5° C. Stubs and isolated naked larvae were exposed, with and without contact moisture, in groups of 50 and 25, respectively, for 5, 10, 15, and 20 days. A control sample of stubs was held at 30° C. and 70% relative humidity, and the experimentals were also held at these conditions following the low temperature treatment. All exposures were unsuccessful in breaking diapause, as not a single larva became prepupal. Later work indicates that the exposure periods were insufficient. In addition, however, the postexperimental treatment was too hot and too dry for satisfactory postdiapause development. Nevertheless, if diapause had been broken, the occasional individual would have developed to at least the prepupal stage at 30° C. and 70% relative humidity. Since this did not occur, the conclusion that exposures of 2° to 5° C. for periods up to 20 days are insufficient to break diapause is substantially correct.

On Sept. 12 and 15, 1944, further exposures were made at temperatures of −15° and −20° C., using larvae from stubs collected on Sept. 9. Larvae were removed from their stubs and exposed in clean honeycomb, after which they were held at 30° C. and 70% relative humidity for observation. At −15° C. larvae were exposed for five hours, one day, three days, and seven days; at −20° C. for three hours, five hours, one day, two days, and three days. There was no development following any of these treatments, and the remarks made above concerning the previous experiment also apply here.

No further experiments were carried on at this time, as no more diapause larvae were available. In the meantime, however, the conditions for re-establishment of diapause were being worked out, and in the spring a collection of stubs was made and exposed to 35° C. for 30 days to provide diapause larvae for further experimentation. A control sample proved to be in diapause; the remainder were held at 5° and 10° C. for periods of 20, 30, 45, and 60 days, followed by an incubation period of 20 days at 28° C. All stubs were kept in moist soil. No visible development occurred in any sample, and it was concluded that the treatments were either ineffective or insufficient. In comparison with a later experiment, in which, however, the diapause was natural and not artificially induced, it appears that the 60 day exposure to 10° C. should have broken diapause in some individuals at least. Since this was not the case, it may indicate a quantitative difference between the fall and spring types of diapause.

Half of the stubs conditioned at 5° and 10° C. for 20 and 30 days were not split open after their incubation period at 28° C., but were reconditioned for an additional 20 days, while the two 45 day samples were given an additional 30 days. This was followed by a second 20 day incubation period, this time at 25° C. Again there was no development.

The only successful breaking of diapause during the 1944-45 season occurred in a small sample exposed to 10° C. for 80 days. These larvae were definitely in artificial diapause beforehand. After an incubation period of 20 days at 25° C. 17 out of 19 specimens showed visible development, 15 being early pupae and two prepupae. The developmental index was 52.

Tests made during the 1945-46 season were much more successful. On Aug. 29, 1945, freshly collected stubs containing larvae in natural fall diapause were placed in soil of 17% moisture content at 5° and 10° C. and in a root cellar. After periods of 40, 50, 60, 70, 80, 90, and 100 days, samples were incubated for 20 days at 25° C. Stubs were then split until 20 living specimens were obtained. Dead larvae were disregarded, as they were either dead before the experiment or else died for reasons not pertinent to the experiment. There was no mortality among later stages (in the stubs), and no parasitism. Following removal from the stubs on the 20th day of incubation, the specimens were placed in 1 in. lengths of 1.5 to 2.0 mm. glass tubing, at 25° C., for further observation. As explained above, this method proved fairly satisfactory for individuals that had already begun postdiapause development, but not for those that had not. The best comparison among samples is therefore obtained on the 20th day at 25° C. when the stubs were split. However, comparisons can also be made of subsequent development, as the microclimatic conditions within the tubes were presumably similar. Tables V, VI, and VII list the developmental data; in the case of the 5° C. series, the time was extended to include samples at 140 and 150 days.

Comparing the three tables with each other it is apparent that diapause was eliminated much sooner at 10° C. than at 5° C., and somewhat sooner at 10° C. than in the root cellar. This comparison, it should be noted, is on the basis of time only; larvae held at 5° C. came out of diapause just as well as those at 10° C., but the conditioning period required was considerably longer. It appears probable that the time required at temperatures below 5° C. is still longer, which would explain the lack of success in the experiments previously discussed, where temperatures of 2° to 5°, $-15°$, and $-20°$ C. were used. The optimum temperature, or the one at which diapause is broken most rapidly, must lie not far from 10° C. Not far above 10° C. lies the developmental range, in which diapause cannot be broken by temperature.

The effects of low temperature on diapause are best seen by the data in Table VI. A period of 40 days at 10° C. was insufficient to break the diapause

TABLE V

PERCENTAGE OF INDIVIDUALS DEVELOPING, AND DEVELOPMENTAL INDICES (*D.I /x*, WHERE *x* EQUALS THE NUMBER OF SPECIMENS ULTIMATELY DEVELOPING) AFTER CONDITIONING DIAPAUSE *Cephus cinctus* LARVAE AT 5° C. FOR 40 TO 150 DAYS

Days at 25° C.	Days at 5° C								
	40-80[1]	90		100		140		150	
		A[2]	*B*[3]	*A*	*B*	*A*	*B*	*A*	*B*
20		10	40	20	50	95	54	100	65
21		—	—	—	—	95	56	100	70
22		10	40	20	55	95	60	—	—
23		—	—	—	—	—	—	100	84
24		—	—	20	55	95	68	100	90
25		10	50	—	—	—	—	—	—
26		—	—	20	60	95	78	100	94
27		10	50	—	—	95	81	—	—
28		—	—	—	—	95	85	100	97
29		—	—	20	75	95	88	—	—
30		10	50	—	—	—	—	100	99
31		—	—	20	85	95	93	100	100
32		10	70	—	—	—	—		
33		—	—	20	90	95	97		
34		10	90	—	—	95	99		
35		—	—	—	—	95	100		
36		10	100	20	95				
37				—	—				
38				20	100				

[1] *No development*

[2] *A = percentage developing*

[3] *B = D I /x*

of any larva. It was broken in 38% after 50 days at 10° C ; 70% after 60 days; 85% after 70 days; 80% after 80 days; 95% after 90 and 100 days. There are minor irregularities, but in general the increase is proportional to the conditioning period An exposure of 110 days at 10° C. would no doubt have been effective in all cases It is apparent, however, that the time required to break diapause varies considerably among individuals. Some required fewer than 50 days at 10° C ; a few more than 90 days; and the majority from 60 to 80 days Thus it is not correct to speak of diapause being gradually broken during the conditioning period if reference is made to individuals; in a group, however, this is correct.

The progress of diapause elimination under outside conditions may be followed in Table VIII, which lists the developmental indices and the percentage of developing individuals in samples of infested stubs brought into the laboratory from the field at weekly intervals from Oct. 5, 1945, to March 1, 1946. Each sample was incubated at 25° C. for 20 days, in soil of 17% moisture content.

The progressive elimination of diapause shown in Table VIII is essentially the same as that in Tables V, VI, and VII, but it is more complete, and in

TABLE VI

PERCENTAGE OF INDIVIDUALS DEVELOPING, AND DEVELOPMENTAL INDICES (*D.I./x*, WHERE *x* EQUALS THE NUMBER OF SPECIMENS ULTIMATELY DEVELOPING) AFTER CONDITIONING DIAPAUSE LARVAE OF *Cephus cinctus* AT 10° C. FOR 40 TO 100 DAYS

Days at 25° C.	Days at 10° C.													
	40[1]	50		60		70		80		90		100	
		A[2]	*B*[3]	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*
20		0	0	10	6	50	25	75	39	85	47	95	56
21		—	—	—	—	—	—	—	—	—	—	—	—
22		0	0	—	—	—	—	—	—	90	54	95	57
23		—	—	—	—	—	—	80	53	—	—	—	—
24		0	0	—	—	60	38		—	—	—	95	65
25		—	—	25	14	—	—	80	56	95	63	—	—
26		0	0	—	—	—	—	—	—	—	—	95	74
27		—	—	—	—	70	45	—	—	95	74	—	—
28		8	8	—	—	—	—	80	64	—	—	—	—
29		—	—	—	—	—	—	—	—	—	—	95	81
30		15	16	70	57	—	—	80	74	95	84	—	—
31		—	-	—	—	85	63	—	—	—	—	95	88
32		23	28	—	—	—	—	80	87	95	89	—	—
33		—	—	—	—	85	70	—	—	—	—	95	94
34		23	32	70	71	—	—	—	—	95	97	—	—
35		—	—	—	—	85	78	80	94	—	—	—	—
36		31	48	—	—	—	—	—	—	95	97	95	97
37		—	—	70	86	—	—	80	95	—	—	—	—
38		31	56	—	—	85	79	—	—	—	—	95	100
39		—		—	—	—		—	—	95	99		
40		31	64	-	—	85	85	80	98	—			
41		—	—	70	96	-	—		—	95	99		
42		38	80	—	—	85	89	80	100	—	—		
43		—	—	70	100	—	—			95	100		
44		38	80			—	—						
45		—	—			85	96						
46		—	—			—	—						
47		38	84			85	99						
48		—	—			—	—						
49		—	—			—	—						
50		—	—			85	100						
51		38	88										
52		—	—										
53		38	92										
54		—	—										
55		38	92										
56			—										
57		—	—										
58		38	96										
59		—	—										
60		38	100										

[1] *No development*

[2] *A = percentage developing.*

[3] *B = D.I./x.*

addition it gives some idea of what actually occurs during the fall and winter under natural conditions. For instance, 10% of the larvae were postdiapause by Oct. 19, 1945, and 25% by Nov. 2. The intervening sample, with 65% postdiapause larvae on Oct. 26, is greatly out of line with the other samples.

The only explanation offered is that of mere chance, as a result of the small size of the sample (20 specimens). Diapause was broken in more and more specimens throughout the winter, and was complete by the end of January, 1946.

TABLE VII

PERCENTAGE OF INDIVIDUALS DEVELOPING, AND DEVELOPMENTAL INDICES (*D.I./x*, WHERE *x* EQUALS THE NUMBER OF SPECIMENS ULTIMATELY DEVELOPING) AFTER CONDITIONING DIAPAUSE LARVAE OF *Cephus cinctus* IN A ROOT CELLAR FOR 40 TO 100 DAYS

Days at 25° C.	Days in root cellar														
	40[1]	50		60		70		80		90		100	
		A[2]	*B*[3]	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*
20		0	0	10	6	5	4	60	31	65	36	55	41
21		—	—	—	—	—	—	—	—	—	—	—	—
22		—	—	—	—	—	—	—	—	75	42	55	43
23		—	—	—	—	—	—	70	44	—	—	—	—
24		—	—	—	—	5	4	—	—	—	—	70	54
25		—	—	20	18	—	—	75	53	80	52	—	—
26		—	—	—	—	—	—	—	—	—	—	70	59
27		—	—	—	—	5	4	—	—	80	61	—	—
28		—	—	—	—	—	—	75	58	—	—	—	—
29		—	—	—	—	—	—	—	—	—	—	70	70
30		—	—	50	40	—	—	75	59	80	66	—	—
31		—	—	—	—	35	33	—	—	—	—	70	77
32		—	—	—	—	—	—	75	69	80	73	—	—
33		—	—	—	—	35	38	—	—	—	—	70	86
34		—	—	55	54	—	—	—	—	85	85	—	—
35		—	—	—	—	35	45	80	82	—	—	—	—
36		—	—	—	—	—	—	—	—	85	89	70	91
37		—	—	60	60	—	—	80	89	—	—	—	—
38		—	—	—	—	45	53	—	—	—	—	70	96
39		—	—	—	—	—	—	—	—	85	95	—	—
40		—	—	—	—	45	62	80	91	—	—	70	100
41		—	-	65	74	—	—	—	—	85	98		
42		—	—	—	—	45	73	80	93	—	—		
43		—	—	65	85	—	—	—	—	85	99		
44		5	40	—	—	—	—	80	95	—	—		
45		—	—	65	88	45	84	—	—	—	—		
46		—	—	—	—	—	—	80	99	85	100		
47		10	70	—	-	45	87	—	—				
48		—	—	65	94	—	—	—	—				
49		—	—	—	—	—	—	80	100				
50		—	—	65	94	45	89						
51		10	80	—	—	—	—						
52		—	—	65	95	45	93						
53		10	80	—	—	—	—						
54		—	—	—	—	45	98						
55		10	80	65	97	—	—						
56		—	—	—	—	45	100						
57		—	—	65	97								
58		10	90	—	—								
59		—	—	—	—								
60		10	90	65	100								
61		—	—										
62		10	100										

[1] *No development.*

[2] *A = percentage developing.*

[3] *B = D.I./x.*

PERCENTAGE OF DEVELOPING INDIVIDUALS AND DEVELOPMENTAL INDICES (*D.I./x*, WHERE *x* EQUALS THE NUMBER OF SPECIMENS ULTIMATELY DEVELOPING) FOLLOWING NATURAL OUTDOOR CONDITIONING. SAMPLES TAKEN WEEKLY FROM OCT. 5, 1945 TO MAR. 1, 1946

Days at 25° C.	Outdoors until:																																							
	Oct. 5[1]	Oct. 12[1]	Oct. 19		Oct. 26		Nov. 2		Nov. 9		Nov. 16		Nov. 23		Nov. 30		Dec. 7		Dec. 14		Dec. 21		Dec. 28		Jan. 4		Jan. 11[4]	Jan. 18[4]	Jan. 25		Feb. 1		Feb. 8		Feb. 15		Feb. 22		Mar. 1	
			A[2]	*B*[3]	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*			*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*
20			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	40	25	55	32	60	28	70	35			90	47	90	46	100	47	95	58	100	58	100	63
21			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	80	40			95	50	90	48	100	52	95	59	—	—	—	—
22			—	—	—	—	—	—	—	—	0	0	0	0	0	0	0	0	60	38	60	36	70	38	80	45			95	50	90	50	100	58	95	63	100	65	100	76
23			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	85	52			95	54	90	53	—	—	—	—	—	—	—	—
24			—	—	—	—	—	—	0	0	0	0	0	0	33	16	23	15	65	46	70	49	75	44	85	53			95	55	95	58	100	61	95	66	100	75	100	93
25			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			95	55	95	58	—	—	—	—	—	—	100	96
26			—	—	—	—	—	—	—	—	0	0	0	0	—	—	41	25	65	51	75	52	75	48	—	—			95	57	95	63	100	67	95	75	100	88	—	—
27			0	0	—	—	—	—	—	—	—	—	—	—	61	29	—	—	—	—	—	—	—	—	85	59			100	61	95	67	100	69	95	85	—	—	100	100
28			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	85	55	85	63			100	66	95	74	100	80	—	—	—	—		
29			—	—	—	—	—	—	0	0	10	8	0	0	78	44	59	40	65	60	75	61	85	58	85	67			100	72	95	79	100	82	95	94	100	98		
30			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	85	61	85	75			100	76	—	—	—	—	—	—	—	—		
31			—	—	—	—	0	0	5	7	10	8	5	5	78	47	59	45	65	66	75	69	85	65	85	76			100	84	95	91	100	94	95	99	100	100		
32			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	85	82			100	90	—	—	—	—	—	—				
33			—	—	—	—	—	—	5	7	40	36	—	—	83	55	65	53	65	75	75	76	—	—	85	89			100	92	95	96	100	99	95	100				
34			—	—	—	—	—	—	—	—	—	—	25	25	—	—	—	—	—	—	—	—	85	80	85	94			100	95	95	99	100	100						
35			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	75	87	85	84	85	95			100	97	95	100								
36			—	—	—	—	10	16	5	10	40	40	25	27	83	60	65	60	65	85	75	89	85	87	85	96			100	97										
37			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	75	93	85	93	85	98			—	—										
38			—	—	65	49	20	40	5	10	40	48	25	35	83	68	65	67	65	94	75	96	85	95	85	100			100	98										
39			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	85	97					—	—										
40			—	—	—	—	25	48	15	23	40	52	30	40	83	75	65	74	65	97	75	100	85	98					100	99										
41			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			85	100					—	—										
42			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	65	100									100	100										
43			—	—	65	62	25	56	25	43	40	52	35	50	83	88	65	84																						
44			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—																						
45			10	40	65	63	25	60	25	43	40	60	35	60	83	93	65	89																						
46			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—																						
47			—	—	65	72	25	68	—	—	50	76	40	67	83	96	65	96																						
48			—	—	—	—	—	—	30	57	—	—	—	—	—	—	—	—																						
49			—	—	—	—	—	—	—	—	—	—	—	—	—	—	65	98																						
50			10	60	65	88	25	80	30	60	50	84	40	80	83	100	—	—																						
51			—	—	—	—	—	—	—	—	—	—	—	—			65	100																						
52			10	60	69	91	25	88	30	60	50	96	40	82																										
53			—	—	—	—	—	—	—	—	—	—	—	—																										
54			10	60	65	97	—	—	30	67	50	96	40	90																										
55			—	—	—	—	25	100	—	—	—	—	—	—																										
56			—	—	—	—			—	—	—	—	—	—																										
57			10	70	65	97			30	77	50	100	40	92																										
58			—	—	—	—			—	—			—	—																										
59			10	80	65	97			30	80			40	97																										
60			—	—	—	—			—	—			—	—																										
61			10	80	—	—			30	83			40	100																										
62			—	—	65	100			—	—																														
63			—	—					—	—																														
64			10	90					30	93																														
65			—	—					—	—																														
66			10	90					30	93																														
67			—	—					—	—																														
68			—	—					30	100																														
69			10	100																																				

[1] *No development.* [2] *A = percentage developing.* [3] *B = D.I./x.* [4] *No samples.*

Apart from the progressive elimination of diapause, Tables V to VIII have one other feature in common. As the conditioning period lengthened, not only did the percentage of developing specimens increase, but the time required for these specimens to begin developing steadily decreased. In other words, the longer the exposure at the conditioning low temperature, the *sooner* development starts at the higher temperature, within limits. This can be traced most accurately by comparing the developmental indices (*D.I./x*, where *x* equals the number of specimens ultimately developing) on the 20th day of incubation, the day on which the stubs were split. These data are brought together in Tables IX and X. In each case the developmental index steadily increased as the low-temperature conditioning period lengthened. The limit to this process, for there must be one, has not as yet been determined.

TABLE IX

Developmental indices and percentage developing on the 20th day of incubation only. Data from Tables V, VI, and VII

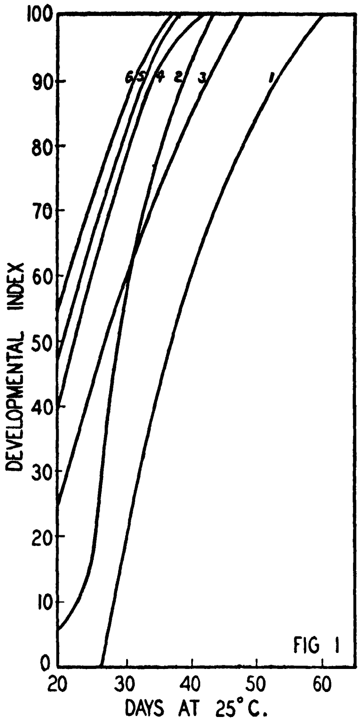
Number of days	5° C		10°C		Root cellar	
	% Developing	*D.I./x*	% Developing	*D.I./x*	% Developing	*D.I./x*
40	0	0	0	0	0	0
50	0	0	0	0	0	0
60	0	0	10	6	10	6
70	0	0	50	25	5	4
80	0	0	75	39	60	31
90	10	40	85	47	65	36
100	20	50	95	56	55	41
140	95	54			—	
150	100	65				—

TABLE X

Developmental indices and percentage developing on the 20th day of incubation only. Data from Table VIII

Date	% Developing	*D.I./x*	Date	% Developing	*D.I./x*
Oct. 5, 1945	0	0	Dec. 21, 1945	55	32
12	0	0	28	60	28
19	0	0	Jan. 4, 1946	70	35
26	0	0	11	—	—
Nov. 2	0	0	18	—	—
9	0	0	25	90	47
16	0	0	Feb. 1	90	46
23	0	0	8	100	47
30	0	0	15	95	58
Dec. 7	0	0	22	100	58
14	40	25	Mar. 1	100	63

For convenience, the data in Table VI and most of those in Table VIII are presented graphically in Figs. 1 and 2, respectively. The developmental curves have been smoothed somewhat and in Fig. 2 every second curve of the earlier samples has been omitted to avoid confusion caused by overlapping.



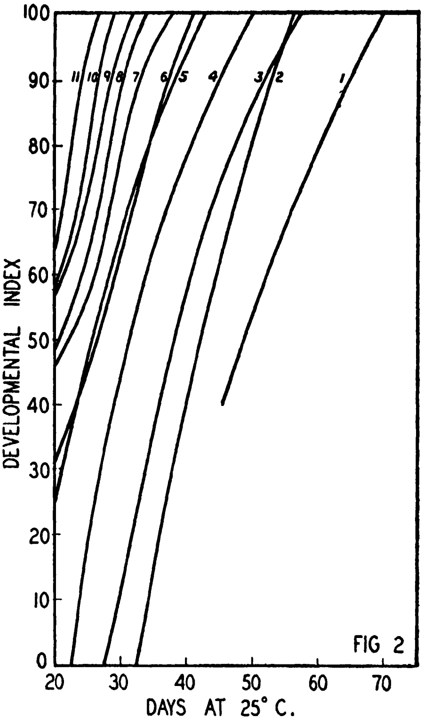


FIG. 1. *Development of Cephus cinctus at 25° C. following various conditioning periods at 10° C.*

1. 50 days at 10° C.	*4 80 days at 10° C.*
2. 60 " " "	*5. 90 " " "*
3. 70 " " "	*6. 100 " " "*

No development after 40 days at 10° C.

FIG. 2. *Development of Cephus cinctus at 25° C. after being brought indoors from the field at various dates.*

1. Oct. 19, 1945.	*7. Jan. 25, 1946.*
2. Nov. 2, "	*8. Feb. 8, "*
3. Nov. 16, "	*9. Feb. 15, "*
4. Nov. 30, "	*10. Feb. 22, "*
5. Dec. 14, "	*11. Mar. 1, "*
6. Dec. 28, "	

No development in samples brought indoors previous to Oct. 19, 1945.

Both figures demonstrate quite clearly the fact that as the period of low-temperature conditioning is increased, the time required to begin postdiapause development is decreased. It is also clearly apparent from the similar slope of the curves that the *rate* of development, after it has once begun, is rather constant. Thus there was no acceleration of development itself; instead there-was a shortening of the postdiapause but predevelopmental period. A discussion of this is given in the next section.

Discussion

There is a critical period in the life cycle of *Cephus cinctus* when diapause can recur after having once been broken. Under natural conditions this period occurs in the spring, following the breaking of the obligatory fall diapause, but preceding active spring development (i.e. before the appearance of the prepupa). For this reason it is called spring diapause to distinguish it from the obligatory fall diapause. Under laboratory conditions spring diapause is readily produced at high temperatures (35° C.). There is good evidence that insufficient moisture at this critical period also reinduces diapause, but this subject is reserved for a later paper.

Although the matter has not been especially investigated, there seems to be no evidence of any qualitative difference between fall and spring diapause. There is slight evidence of a quantitative difference, in that the low temperature conditioning periods required to break the two diapauses may differ, but this is not at all certain.

A temperature of 35° C. has no apparent ill effect in itself on larvae in diapause. It has definite ill effects on developing forms such as prepupae and pupae, in which it produces coma, abnormalities, and death. It reinstates diapause only during the intervening stage, that of the semidiapause S-larva. In all of the present experimental work this stage has been delimited only by indirect means. By conditioning larvae for an ample period at low temperatures the breaking of diapause is effected, and the larvae have been assumed to be postdiapause from the instant they are transferred from the low to the incubation temperature. The S-larval stage has been considered as ended only when the prepupa becomes visually recognizable. This practice is obviously not precise, as the morphological changes distinguishing prepupa from S-larva require some time. Just how much time is actually required is unknown, but since this is a critical period in the life cycle it is of considerable practical importance. The course of the respiratory rate during this crucial period has not been followed as yet, but plans have been made for such a study. It is likely to give a more clear-cut separation of the various stages, especially if diapause in *Cephus cinctus* proves to be similar to that in eggs of *Melanoplus differentialis*. Bodine (1) found that during diapause the respiratory rate of *M. differentialis* eggs is depressed. When postdiapause development begins, the rate rises appreciably, and thus can be used as a test for the presence or absence of diapause. If *Cephus cinctus* reacts similarly, the respiratory rate would rise, at favourable temperatures, as soon as diapause is *completely* eliminated. This point would then mark the true beginning of the prepupal stage, and in addition it would mark the end of the insect's ability to return to diapause.

We have seen, in this connection, that the insect does not, in most instances begin to develop as soon as it is brought to the incubation temperature. It has been shown in Tables V to VIII and Figs. 1 and 2 that as the exposure to the conditioning low temperature is increased, the time until development starts, when measured by the visible change to the prepupa, is decreased. Since some individuals may become prepupal as much as 20 days (at 25° C.) earlier than others, it is apparent that all do not begin to develop at the same time. The difference must occur in the predevelopmental stage here called the "semidiapause S-larva".

The assumption has been made that if the exposure to a low temperature is sufficient to allow postdiapause development at the incubation temperature, diapause is 'broken'. Thus all individuals listed in Tables V to VIII that developed (i.e. reached the prepupal stage) would be 'out of diapause', regardless of the time required for them to start to develop, or reach the prepupal stage. In terms of Bodine's (1) theory of diapause, their x factor was eliminated or reduced below the level of effectiveness. Yet it has been shown above that after the x factor is inoperative the beginning of postdiapause development must wait for something else—presumably another physiological reaction. Following Bodine's analogy, we can postulate a y factor, which remains to be eliminated after x has gone. But whereas x is eliminated only at low temperatures, y is eliminated at both low and high temperatures. Thus it may be correct to say that diapause is 'broken' after the x factor has been made inoperative, but it is not 'eliminated' until after the y factor has also been made inoperative.

To take a definite example from Table VI, in the sample of larvae conditioned at 10° C. for 50 days the most tardy individual became prepupal on the 41st or 42nd day of incubation at 25° C. In the sample conditioned at 10° C. for 90 days, the most tardy individual became prepupal on the 23rd, 24th, or 25th day. In the additional 40 days at 10° C. the y factor was reduced to the same extent as in 16 to 19 days at 25° C. The reduction in time in each case represents a reduction in the y factor. Even in the latter sample the y factor was far from eliminated after 90 days at 10° C. In Table VIII there is evidence that the y factor was not quite eliminated by Mar. 1, 1946. The time at 25° C. required for all ultimately developing specimens to start developing was gradually being reduced. Just where the minimum lay was not determined because of difficulties in the rearing technique that occurred when the larvae had to be reared apart from their protective stubs and cocoons. This point, however, will be investigated later by the use of a respirometer.

A question of terminology naturally arises over the use of the term 'postdiapause'. As soon as the x factor has been eliminated, diapause is broken (though not eliminated, for the y factor remains) and the insect still has the appearance of an S-larva. It has been stated above in this discussion that it is probable that the beginning of increased respiratory rate, the beginning of the prepupal stage, and the end of the ability to return to diapause all fall at the same time. Now, "the end of the ability to return to diapause" may be changed to "the elimination of the y factor" with the understanding that diapause cannot be reinstated when both x and y factors are gone. The normal course of events in *Cephus cinctus* would then be as follows. Beginning with an S-larva in diapause, the *x* factor is dissipated at low temperatures. Following this the *y* factor is dissipated at either low or moderately high temperatures. As soon as the *y* factor is completely gone, diapause is over, postdiapause development begins, and the insect is then a prepupa, though it will continue to look like an S-larva for a short time. No ecdysis marks either this or the previous change. Until these various stages or phases are more exactly delimited by means of physiological tests we have a curious mixture of morphological and physiological steps, which may be summarized as follows:

	Morphological form	Physiological form
1. Diapause S-larva (*x* and *y* factors operative)	Larva (non-developing)	Larva (non-developing)
2. Semidiapause S-larva (*y* factor, only, operative)		
3. Postdiapause S-larva = S-prepupa		Prepupa (developing)
4. Prepupa	Prepupa (developing)	

Although, as stated, there is a lack of exact delimitation of the above stages, there is no doubt about their existence. Nor are they of merely theoretical interest. The semidiapause S-larva is readily returned to full diapause and is thus of considerable practical importance. It is of interest to note in this regard that the species has a double protection against postdiapause development in the fall. A small percentage of the larvae had their diapause broken (*x* factor only) on Oct. 19, 1945, but it required between 28 and 45 days at 25° C. for them to start development (Table VIII). In the cooler temperatures of their natural environment, even allowing for periods of comparative warmth, it would be impossible for them to start development before winter. By the time that temperatures are high enough in the spring to permit development, the *y* factor will have been entirely or nearly eliminated, thus wiping out individual differences in *x*-factor elimination.

If it is thought of as a reaction similar to Bodine's *x* factor, the *y* factor is reduced at a faster rate at 25° C. than at 10° C. or 5° C. Although the full range was not specifically investigated, it seems probable that the rate of elimination increases with temperature within limits bounded by the undercooling point on the one hand and a temperature of about 30° C. on the other. (Reinstatement of diapause starts at a temperature of about 30° C. in many cases. Some *y* factor still remains in such specimens.) The *x* factor was eliminated faster at 10° C. than at 5° C., and presumably faster at 5° C. than at lower temperatures. Again, the rate of elimination increased with temperature within limits, this time by the undercooling point on the one hand and a temperature not much above 10° C. on the other. The low upper limit in the case of the *x* factor reaction does not seem to be readily explainable with only the facts already known. If a conjecture is permissible, it would seem that the *x* factor reaction is in some way interfered with at developmental temperatures. The threshold of development, or at least the threshold of certain developmental processes, lies not far above 10° C., and this is approximately where the upper limit of the *x* factor reaction lies.

The terms "*x* factor" and "*y* factor" are used here more for convenience than anything else. Their nature is at present quite vague and uncertain even though there are a few things we can definitely say of them. We know, for instance, the order of their occurrence or elimination and their reactions to a rather wide range of temperatures. We also know that they are reversible, because a return to full diapause is readily accomplished under certain conditions. This latter fact rules out the possibility of morphological changes being associated with the process of diapause elimination, since morphological changes are not reversible. It seems very likely that the process is chemical, though whether enzymatic, hormone-controlled, or otherwise is at present uncertain.

It has been shown that earliness of postdiapause development in *Cephus cinctus* is related to size. Large larvae generally reach the prepupal and pupal stages well in advance of similarly conditioned small larvae. The difference does not lie in the rate of postdiapause development, but in the time prior to the beginning of this development. Again, it is the semidiapause S-larval stage that varies, and we are faced with the probability that in large larvae the *y* factor is eliminated sooner (possibly also faster) than in small larvae. No attempt is made here to explain why this is so, but the fact that it is so accounts for the rather wide range of development within samples. This variability was the cause of much concern during the earlier part of this experimental work. It was originally planned to set up standard rearing conditions as a basis for comparison, with the expectation that postdiapause development would be rather uniform. While development following the visibly-recognizable prepupal state was reasonably uniform (Figs. 1, 2), the earlier period was far from it. This led to a recognition of the relation of size to *y*-factor elimination, and to the abandonment of the attempt to set up standard conditions, since the stub prevents selection of samples uniform as to size.

Acknowledgments

The writer wishes to express his appreciation to Dr. C. W. Farstad of the Dominion Entomological Laboratory at Lethbridge, Alta., and Dr. J. H. Pepper of the Montana Agricultural Experiment Station for helpful criticism and advice, and to the former also for permission to use unpublished material.

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THE PROVENTRICULUS OF THE LARVA OF THE CADDIS-FLY, *MACRONEMA ZEBRATUM* HAGEN (TRICHOPTERA: HYDROPSYCHIDAE)[1]

By W. W. Judd[2]

Abstract

The structure of the proventriculus of the larva of *Macronema zebratum* Hagen is described. The form of the organ is similar to that of some other larvae of the Hydropsychidae. It probably functions as a masticator or compressor of food.

Introduction

The structure of the digestive tract of caddis-fly larvae has been studied by a few authors who note a variety of patterns in the arrangement of folds on the sclerotized lining of the proventriculus. Lloyd (4) reports that the later instars of the larvae of the Hydropsychidae are carnivorous and Glasgow (3) reports that the proventriculus of *Hydropsyche colonica* is characterized by 34 to 38 strong, sclerotized teeth provided with small denticles and by great development of the circular muscles. Lloyd (4) and Gatjen (2) state that many of the larvae of the Phryganeidae are herbivorous and that the proventriculus of *Phryganea interrupta* Say in this family (1) is large and has a heavy musculature In *Anabolia laevis* Zett. Russ (5) shows that the sclerotized lining of the proventriculus is thin and the musculature weak except where the proventriculus joins the oesophageal valve.

Materials

Larvae of *Macronema zebratum* Hagen were collected from the lower surface of stones in shallow water of the Ottawa River at Ottawa, Ont., Sept. 2, 1939. They were identified by Dr. T H Frison, Chief of the Illinois Natural History Survey, Urbana, Ill

The digestive tracts were removed from specimens and pinned to a layer of wax in a Syracuse watch glass and then covered with water The proventriculus was cut longitudinally with fine scissors A section including the proventriculus was cut from the digestive tract and was placed for 12 hr. in a solution of potassium hydroxide, which dissolved the muscles and other soft tissues. The sclerotized lining of the organ, divested of muscles, was passed through several changes of water, alcohols, and xylol and then mounted in Canada balsam.

Serial transverse sections (10μ) were made of a number of digestive tracts and were stained with haematoxylin and eosin.

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[1] *Manuscript received December 19, 1946*
Part of a thesis presented in accordance with the requirements for the degree of Master of Arts of the University of Western Ontario, London, Ont

[2] *At the time, Demonstrator in Biology, University of Western Ontario, now, Lecturer in Zoology, McMaster University, Hamilton, Ont*

Description

The proventriculus of the larva of *Macronema zebratum* Hagen is 2 mm. long and lies in the mesothorax or metathorax. It consists of two regions separated by a constriction. The anterior region is 0.75 mm. broad anteriorly and tapers posteriorly toward the constriction. The posterior region is cylindrical and 0.15 mm. broad.

The outer layer of the anterior region consists of heavy circular muscle 0.1 mm. thick (Figs. 3, 4–CM). Next to the circular muscle is the layer of epidermal cells (Fig. 3–EP). Between the epidermal layer and the lumen of the proventriculus is the sclerotized intima. This is organized in the form of 18 longitudinal teeth running the length of the anterior region (Figs. 1, 2, 3, 4–LT). These teeth are flattened laterally (Fig. 3) and their greatest height is attained at points about three-quarters of the length from their anterior ends (Fig. 2). Where their peaks converge they almost block the lumen of the proventriculus (Fig. 4).

In the posterior region of the proventriculus the circular muscle is about 0.05 mm. thick (Figs. 5, 6, 7–CM). Corresponding with the 18 teeth of the anterior region there are in the posterior region 18 longitudinal folds (Figs. 2, 5–LF) that extend posteriorly about one-quarter of the length of the posterior region. They are beset with a heavy coat of strong hairs that converge into the lumen (Figs. 1, 2, 5–CS). Posterior to the longitudinal folds, and bulging downward from the dorsal wall of the proventriculus, is a sclerotized pad (Figs. 1, 6–P) beset with small spines and almost filling the lumen. At each side of the pad there is a longitudinal ridge (Figs. 1, 6–LR). Posteriorly the pad and ridges become laterally flattened and in transverse section appear as three processes projecting downward across the lumen (Fig. 7–P, LR). Small longitudinal folds appear along those parts of the sides of the lumen not occupied by the pad and longitudinal ridges.

The foregoing description of the structure of the proventriculus of the larva of *M. zebratum* indicates that this organ is similar to that of other members of the Hydropsychidae. Branch (1) shows that in one species of *Hydropsychodes* the proventriculus has 36 heavy 'stomachic' teeth; and Glasgow shows that in one species of *Hydropsyche* there are from 34 to 38 strong teeth. It is probable that this organ functions as a masticator or

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FIGS. 1–7. *Figures of the proventriculus of Macronema zebratum showing the flattened sclerotized lining and transverse sections at several levels.*

CM: circular muscle; CS: sclerotized hairs; EP: epidermal cells; LF: longitudinal fold; LR: longitudinal ridge; LT: longitudinal tooth; P: sclerotized pad.

FIG. 1. *Part of the sclerotized lining of the proventriculus showing four longitudinal teeth.*
FIG. 2. *Lateral view of longitudinal tooth.*
FIG. 3. *Transverse section through anterior ends of teeth.*
FIG. 4. *Transverse section through peaks of teeth.*
FIG. 5. *Transverse section through sclerotized folds.*
FIG. 6. *Transverse section through anterior end of sclerotized pad.*
FIG. 7. *Transverse section through posterior end of sclerotized pad.*

Fig. 1.

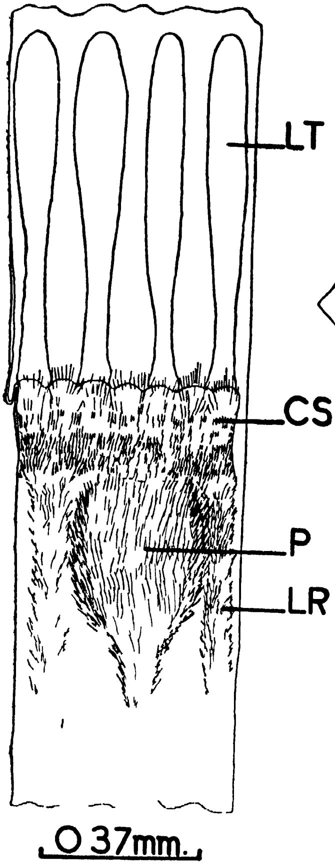


Fig. 2.

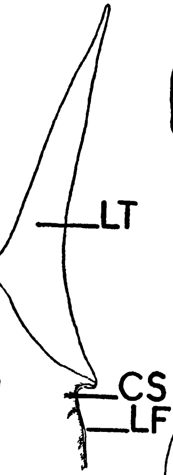


Fig. 3.

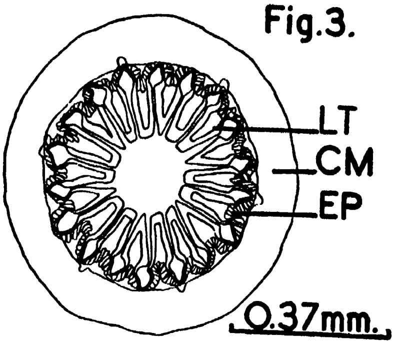


Fig. 4.

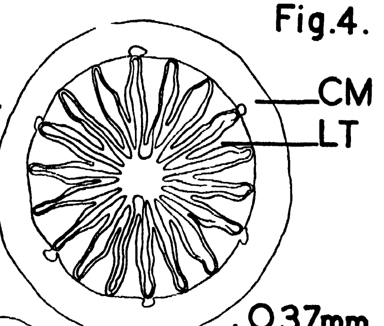


Fig 5.

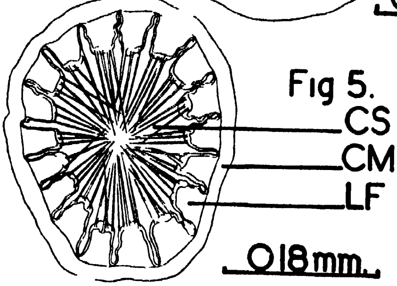


Fig. 6.

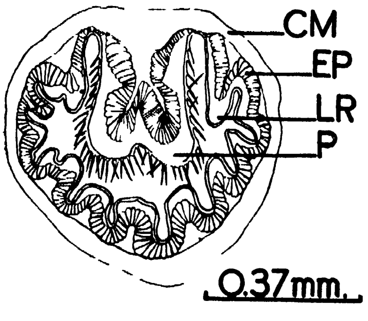
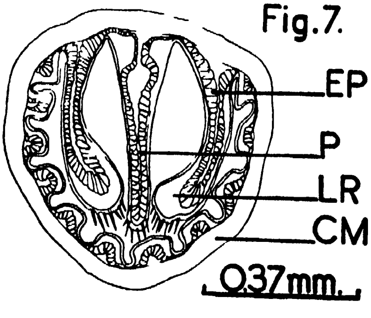


Fig. 7.



compressor of food. Lloyd (4) states that the larvae of this family do not chew their victims, but grasp them with their forelegs and thrust them bodily into their alimentary cavity. It would consequently be advantageous for the insect to have a well developed proventriculus for chewing or compressing the ingested insect.

Acknowledgment

The writer wishes to express his gratitude to Prof. J. D. Detwiler of the University of Western Ontario for his guidance in this work.

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THE EFFECT OF TEMPERATURE ON THE RATE AND VARIATION OF OPERCULAR MOVEMENT IN *FUNDULUS DIAPHANUS DIAPHANUS*[1]

By Benjamin N. Kropp[2]

Abstract

The rates of opercular beat of 16 specimens of *Fundulus diaphanus diaphanus* were recorded over a temperature range from 4 3° to 17 5° C. in order to determine how this respiratory movement varied with temperature and some of the sources of variation in rate. While the rate of beat varies directly as the temperature, over a period of several hours at any constant temperature continuous recordings of the rate show recurring cycles of rise and fall in beat frequency that are chiefly responsible for the scatter of the observations. Both the duration of a cycle and the limits of rise and fall for each cycle are definitely set by the temperature. The possible dependence of these phenomena upon central nervous activity is discussed.

The rate of respiratory movements in poikilothermic animals, and in newborn mammals before the thermoregulatory capacity has been developed, usually varies directly with the environmental temperature (Stier and Pincus (5), Sumner (6)). At any constant temperature the observed rate of movement of operculum, gill, or other organ in such animals varies within limits determined by the particular function and temperature (cf. Fig. 1). In the work here reported the attempt was made to determine how respiratory movements in *Fundulus diaphanus diaphanus*, as measured by the observed rate of beat of the operculum, varied with temperature, and some of the sources of variation in rate.

The animals used in this study were collected during August and September. Each animal was kept in running tap water for at least 24 hr. after entering the laboratory, and before being placed in the thermostat for observation. No selection as to size was subsequently made since in preliminary experiments the opercular response to temperature was found to be independent of the size of the fish. Observations were never made on animals that had been in the laboratory for more than four days. The experimental animals were placed in a Fisher absorption cell suspended in a water-bath thermostat with a slow stream of air, which had been brought to the water-bath temperature, bubbling through the chamber at a constant rate. After each change of temperature at least 20 min. were allowed to permit thermal adaptation before readings were resumed at the new temperature. The use of several stop watches made possible the recording of consecutive beats during the period of observation. The following data are based on observations on 16 animals.

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[1] *Manuscript received February 7, 1946.*

Contribution from the Department of Anatomy, Queen's University, Kingston, Ont. With financial assistance from the Committee on Scientific Research of Queen's University.

[2] *Assistant Professor of Embryology.*

Stop watch readings of the time for 10 opercular beats were made at six different temperatures from 4.3° to 17.5° C. When the average times of 50 readings at each temperature are plotted against temperature (Fig. 1) the points fall along a straight line although there is a marked and progressive divergence from this relationship at lower temperatures. When the extremes of variation above and below the average for each point recorded are shown on the same curve (vertical lines), it is seen that these variations also tend to become progressively greater at lower temperatures and decrease progressively as the temperature is increased. Many previous investigators have noted the very wide variations that occur in the respiratory rate of cold-blooded animals and have sought to increase the accuracy of their data by recording 'large' numbers of readings, usually 30 to 40, at each temperature. It is apparent that in this particular process the method of averages from relatively few readings at each temperature may indicate trends and may even show occasional extreme rates, but gives limited information regarding the relationship of opercular beat to temperature, and no insight either into the process controlling the opercular beat or the nature and variation of the temperature effect. Therefore, to expand the limits of the method, and in order to include all the extreme rates, readings were made for several thousand consecutive beats over the temperature range under investigation. This was done by continuous stop watch readings of the time for 10 opercular beats, taken with the aid of several stop watches and assistants to record the readings (3). Occasional spontaneous movements prevented obtaining completely successive readings, but except when they were particularly violent these movements did not affect the frequency of opercular beat.

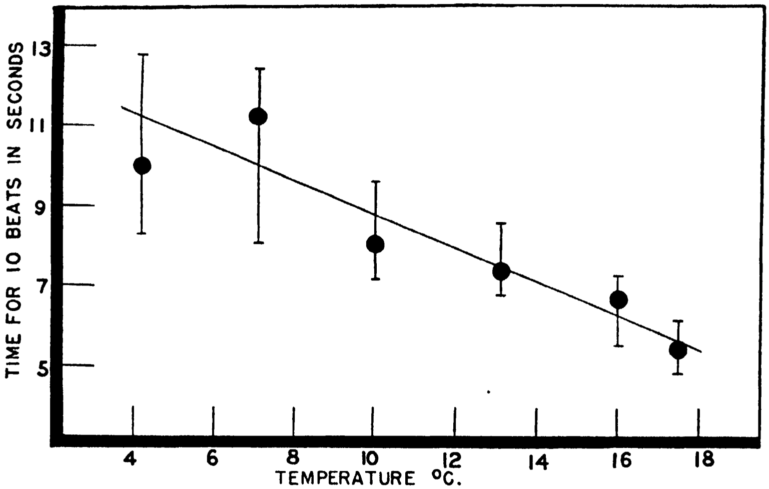

FIG. 1. *Time for 10 beats in seconds at six temperatures from 4.3° to 17.5° C. Average of 50 readings at each temperature. Vertical lines represent limits of variation from each average. Animal No. 2. See text.*

It was found that whenever the temperature of the medium was changed there was an almost immediate rise in the frequency of opercular beat whether the temperature was raised or lowered. There followed a fairly regular decline and rise in the rate of beating, which is illustrated by the data plotted in Fig. 2. The variation or scatter of the points did not occur more or less symmetrically about an average that was characteristic for each temperature. For the data at 17° (Fig. 2, lower curve) there are four definite and successive cycles shown during which the rate of opercular movement gradually declines. The end of each cycle is marked by an abrupt return to a rapid rate and the beginning of a new cycle. In general, the cycles recur more frequently at higher temperatures than at lower ones. Thus, the data at 7° (Fig. 2, upper curve) show but two cycles although in this case the total elapsed time is greater than at the higher temperatures.

The manner of scatter of the observations is well illustrated in Fig. 3. This represents 50 readings at each of the six temperatures indicated on the curve from 5 5° to 17° C. For purposes of comparison these 50 readings were in every case taken at the beginning of a cycle. It will be noted that the scatter of the observations, as indicated by the progressive narrowing of the band enclosing the readings at each temperature, decreases with rising temperature (cf. Fig. 1). This is obvious not only in the observed difference between average frequencies, but also in the decrease of the deviation of the measurements with increasing temperature.

Discussion

Our knowledge of the fluctuations in the underlying process controlling the beat can in most instances be obtained only by assuming that the measured frequencies of beating is an index of these underlying changes. Hence, the frequency of bursts of efferent impulses was assumed to be directly proportional to the velocity of the underlying reactions. The suggestion has been made that periodic variations in the frequency of processes controlling the beat or movement of organs may account for a number of observed differences in frequency of beat at any constant temperature. With regard to the opercular beat of the goldfish Crozier and Stier (4) suggested that the observed variations might be traced to the method of discharge of central nervous impulses controlling the beat. In the case of processes under nervous control it has been possible to measure the fluctuations in the rate of the presumed controlling nerve impulses, and it has in fact been demonstrated (Adrian (1), Adrian and Buytendijk (2)) that nerve impulses may be transmitted regularly along spontaneously discharging nerves from spontaneously discharging centres. In the latter work rhythmical potential changes lasting one to three seconds in the excised brain stem of the goldfish were recorded, and the duration of these potential changes corresponded well with the actual frequency of opercular respiratory movements of the intact fish. The assumption may be made that these centrally originating variations in potential are transmitted as synchronous bursts of efferent impulses to the respiratory musculature.

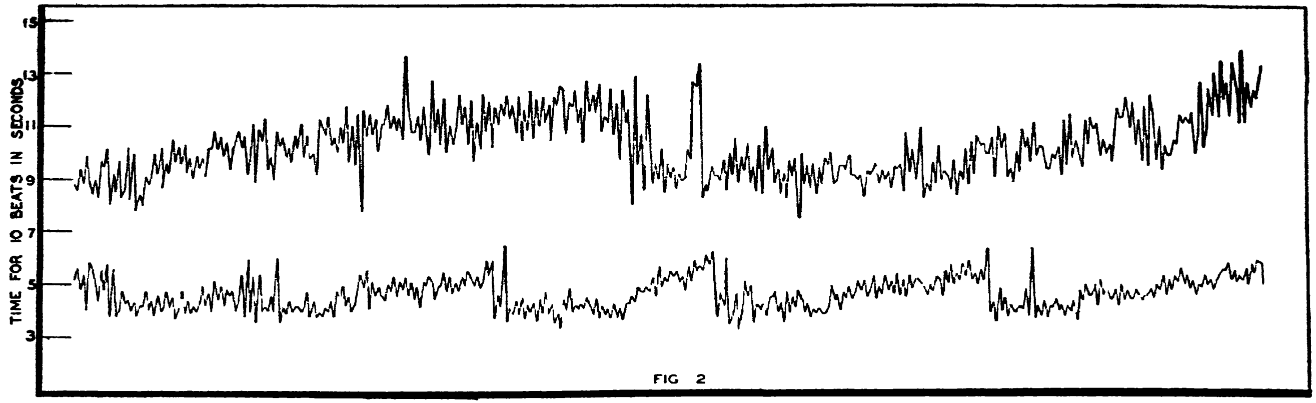

FIG 2

FIG. 2. *Four hundred successive readings (out of several thousand) of time for 10 opercular beats at 7° C. (upper curve) and 17° C. (lower curve). Animal No. 10. See text.*

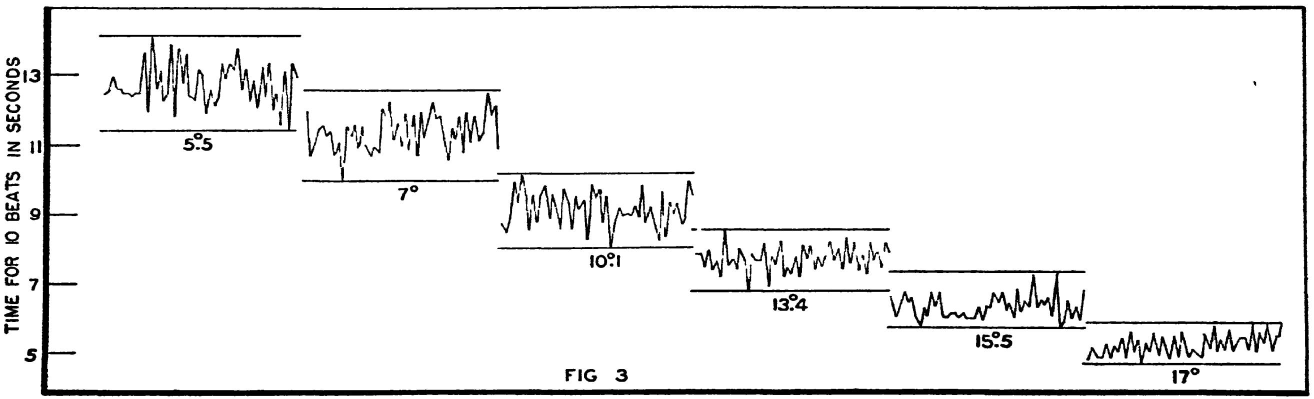

FIG 3

FIG. 3. *Fifty successive readings of time for 10 beats taken at each indicated temperature. Animal No. 6. See text.*

The question arises as to the possible influence on opercular beat of the uncontrolled factor of variation in oxygen consumption, especially since, in studies on *F. parvipinnis*, Wells (7) found the respiratory metabolism to be relatively high at the beginning of his experiments and decreasing with time. This decrease was accompanied by a gradual decrease in oxygen consumption during the first 24 hr. the animals were kept in the laboratory, and thereafter it attained a fairly constant level. Crozier and Stier (4) were unable to find a significant effect upon the breathing frequency of the goldfish after experimentally varying the oxygen concentration from 0.68 to 6.2 cc. per litre. In the present experiments no readings were taken on the opercular beat during the first 24 hr. after the animals were collected, nor were consecutive readings made for as long as a 24 hr. period. However, in observations that lasted as long as six to eight hours on single animals, the gradual decline in rate of opercular beat always ended with an abrupt return to a faster rate and the beginning of a new cycle. There is no reason to believe, therefore, that the gradual slowing of the beat frequency in *F. diaphanus diaphanus* can be accounted for by reduced oxygen consumption, but would seem to be due rather to spontaneous variations of discharge from the respiratory centre.

The possible influence of spontaneous activity on the rate of respiratory movements in the fish is difficult to assess. It occasionally seemed as though the more violent movements may have induced accelerations in rate and the subsequent quiescence was responsible for retardation. But it is more likely that the increase in respiratory rate is of central nervous origin, and the occasional accompanying bodily movements are due to central nervous or external stimulation. Since the described fluctuations in rate, at any temperature, very often take place with very little or no activity of the entire animal it is difficult to see how the activity could be related causatively to the rate.

Acknowledgments

I wish to acknowledge with thanks the assistance in this work of Mr. George H. Clark and Mrs. Laurel C. Kropp.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 25, SEC. D. JUNE, 1947 NUMBER 3

THE CALIFORNIA FLATHEADED BORER (*MELANOPHILA CALIFORNICA* VAN DYKE) IN PONDEROSA PINE STANDS OF NORTHEASTERN CALIFORNIA[1]

BY A. S. WEST, JR.[2]

Abstract

This paper reports the findings of studies on the biology of the California flatheaded borer (*Melanophila californica* Van Dyke). Until comparatively recently this insect had been regarded as a relatively unimportant element of the western pine beetle problem. It has been shown that larval broods may spend a period of up to several years in an incipient stage in the living host tree. During this period the gallery of the incipient stage larva is healed over and the abnormal tissues thus developed interfere with normal conduction in the wood. Undetermined factors associated with the dying of the host tree initiate a fast-growing larval stage in which feeding is voracious and growth rapid.

No attempt is made to set forth the role of the species in the complex known as the western pine beetle problem. It is, however, suggested that this insect, at least in northeastern California, may play a more important role in insect caused losses in commercial stands of ponderosa and Jeffrey pine than had previously been believed. The contributions to the knowledge of the biology of the species may assist in an eventual better understanding of insect-host relationships in this particular problem.

Foreword

The role of the numerous groups of insects attacking forest trees is in many cases complex. In particular the role of flatheaded borers (Coleoptera, Buprestidae) has been in general poorly understood. In part, an understanding of this role has been obscured by the activity of and attention paid to other agencies such as bark beetles of the genus *Dendroctonus* in relation to western pines.

During recent years considerably greater attention has been given to several buprestids, and it is to be hoped that these investigations will lead to a clearer understanding of the host relationships involved.

In general the buprestids that feed on the cambium region have been considered 'secondary' insects, that is, not capable of attacking and killing

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[1] *Manuscript received in original form October 15, 1946, and as revised, March 18, 1947.*

Contribution from the Department of Biology, Queen's University. This paper reports work completed principally during 1935-1939 when the writer was associated with the United States Department of Agriculture, Bureau of Entomology and Plant Quarantine, Division of Forest Insect Investigations. The ideas expressed herein are those of the writer and do not necessarily represent those of his associates in the Division nor in any way relate to the policy of the Division of Forest Insect Investigations as regards western forest insect problems.

[2] *Associate Professor of Zoology, Queen's University; formerly Assistant Conservationist, United States Department of Agriculture, resigned October, 1939.*

[The April issue of Section D (Can. J. Research, D, 25 (2) :45-95. 1947) was issued June 4, 1947.]

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healthy, vigorous trees, but associated with one or more predisposing or weakening factors. Certain species can almost always be shown to be associated with trees that are decadent or dying as the result of a primary or initiating factor. The hemlock borer (*Melanophila fulvoguttata* Harr.) develops in trees (*Tsuga canadensis* (L.) Carr.) whose root systems have been wholly or partially killed by drought (Secrest *et al.*, 12) or trees whose root system has been submerged by high water (Balch, 1). *Melanophila gentilis* Lec., the pine flatheaded borer, is another example. This species develops in windfalls, slash, fire-injured trees, and trees in which the cambium has been injured by abnormally low winter temperatures. Ponderosa pine (*Pinus ponderosa* Dougl.) and Jeffrey pine (*Pinus jeffreyi* Grev. and Balf.) are the principal hosts.

Another group consists of species that cannot regularly be shown to be abundant wholly as the result of some preceding or primary factor. Such species may possibly be assigned something more than a purely passive role in the biological complex involved. Admittedly an unrecognized predisposing factor may exist. The bronze birch borer (*Agrilus anxius* Gory) furnishes one example of this type (Balch and Prebble, 2) and *Melanophila californica* Van Dyke, attacking living ponderosa and Jeffrey pines in northeastern California is another striking example.

The writer has had the opportunity of becoming thoroughly familiar with the problems posed by *M. californica* and *M. gentilis* in California and by *A. anxius* in New Brunswick and in addition has visited field study plots in Wisconsin where *M. fulvoguttata* was being investigated.

No final and complete definition of the role of *M. californica* is attempted in the following paper. However, the writer feels that the work herein reported adequately demonstrates the greater importance of the species than would be indicated by previous attention.

Introduction

The activities of the California flatheaded borer in ponderosa pine forests of northeastern California are of considerable interest because of the association of that species in the biological complex that is commonly referred to as the western pine beetle problem. That association is particularly noticeable in the mature and over-mature commercial stands. It is more evident in the marginal stands but is present in some trees in most of the so-called east-side pine type stands in which the western pine beetle problem is important.

This western pine beetle problem exists in northeastern California because of insect activities associated with the loss of lumber values resultant from the death of merchantable ponderosa pine trees. Several species of insects may be involved, not only over given areas, but in single trees as well. The problem was given its common name more than 30 years ago because the western pine beetle (*Dendroctonus brevicomis* Lec.) had been found in most of the infested dying or dead trees on which stem analyses were made. However, other species of insects also commonly occur in infested trees. From

1931 through 1940 nearly 1700 infested trees were examined to determine what species occurred most frequently, in what portion of the tree they were found, and of what importance the infestations of the several species appeared to be. Western pine beetle broods were found in most of the trees. Maturing broods of the California flatheaded borer (*Melanophila californica* Van Dyke) occurred in 53.3% of the trees. The mountain pine beetle (*Dendroctonus monticolae* Hopk.), the red turpentine beetle (*Dendroctonus valens* Lec.), the Oregon pine engraver (*Ips oregoni* Eichh.), and the emarginate *Ips* (*Ips emarginatus* Lec.) also were involved to some extent, but infestations by those species ordinarily appeared to be of relatively minor importance in the general infestation picture.

Thus it was concluded that the western pine beetle and the California flatheaded borer were the two important entomological factors in the biological complex. It was also concluded that the flatheaded borer was of greater importance than its occurrence in slightly over half the sample would indicate since this species appeared to have successfully *initiated* the sequence of insect activity in 48.7% of all the trees. The western pine beetle followed the flatheaded borer infestations in what appeared to be supplementary, more or less secondary, attacks on many of these trees.

Since 1910 most of the research on the western pine beetle problem has been directed toward securing information on the biology, host relations, and control of *Dendroctonus brevicomis*. The accumulation of considerable valuable information has aided in the development and practice of methods to control infestations by that species.

During the same period the California flatheaded borer had been considered a species of secondary importance and little specific information had been gathered concerning it. The reappraisal of the species as an important entomological factor in the western pine beetle problem made it necessary to secure detailed information concerning its biology, host relationships, and control.

A one year life history for *M. californica* had been generally assumed, but some of the first results of this study created considerable doubt as to the accuracy of that assumption. Although Burke (5) had noted . . . . "small, winding pitchy lines" . . . . in connection with flatheaded borer infestations, their significance as a normal result of infestations in living trees was missed until J. M. Miller, in 1936, linked their occurrence with living *M. californica* larvae that were causing injury to the phloem, cambium, and sapwood of ponderosa and Jeffrey pines.

These preliminary observations, most of which were not in agreement with previous conceptions, emphasized the necessity of an intensive study of the species.

The objective of the work reported in this paper was to secure detailed information. When studies were initiated in 1935 considerable attention was also given to the pine flatheaded borer, *Melanophila gentilis*. It was soon indicated that this species was a minor factor in east-side pine infestations, occurring principally in windfalls, slash, and fire-injured trees. The similarities and differences in habits of *M. californica* and *M. gentilis* have been summarized by West (15).

Since the completion of the studies reported herein some additional investigations have been carried out. These have dealt chiefly with variations in the life history. It is stressed that this paper is primarily concerned with the general and normal biology of the species and its relation to pine stands in northeastern California.

Review of Literature

Most of the literature references to the California flatheaded borer are concerned with the taxonomy of the species or are short, general notes summarizing field observations concerning hosts, biology, and habits. In the taxonomic literature the description of the species by Van Dyke (14) and a revision of the genus *Melanophila* by Sloop (13) are of importance because of their technical description of adult characters.

Of the biological notes in the literature those of Burke (3, 4, 5) are of interest. Burke gives the geographical distribution of the species as including the states of Idaho, Oregon, and California and the hosts as ponderosa, Jeffrey, digger (*Pinus sabiniana* Dougl.), Coulter (*Pinus coulteri* D. Don.), Monterey (*Pinus radiata* D. Don.), and knobcone (*Pinus attenuata* Lemm.) pines.* Concerning the life history he mentions that pupation occurs from March to July and flight from May to August. The occurrence of larval sapwood scars in association with pitch pockets is also recorded. At that time pathologists considered that these scars were caused by lightning. Concerning the importance of the species, Burke states that, ———— "it attacks and kills many second growth trees and assists bark-beetles to kill others". Keen (9, 10) added that the California flatheaded borer is very destructive to healthy, weakened, or slightly injured trees, particularly the old decadent types and those growing on rocky slopes, in fringe type stands, or other situations where soil moisture is deficient. Both Keen (10) and Graham (6, pp. 225-226) mentioned the results of some of the work reported in this paper concerning larval and attack habits of the species.

Aside from these few relatively short notes no other significant published information was found.

Description of Developmental Stages

Six recognizably different developmental stages exist. In addition to the adult, egg, and pupal stage the larval stage can be separated into an incipient, a fast-growing, and a prepupal stage.

Keen (10) lists Douglas-fir (Pseudotsuga taxifolia (Poir.) Britt.) and bigcone spruce (Pseudotsuga macrocarpa (Torr.) Mayr.) as reported hosts but these records could not be verified. It is probable that these two species are not normal hosts for the California flatheaded borer.

Adult (Fig. 1)

The adult has a moderately convex, fairly robust form. Its dorsal surface is brownish-black with from one to three yellow maculations of variable size often occurring on each elytron. Forty per cent of 389 specimens examined had immaculate elytra. The ventral surface is a uniform greenish-bronze colour. The body length ranged from 7 to 11 mm., averaging 9.7 mm. for 231 measured females and 8.8 mm. for 238 measured males. In both sexes over 75% of the individuals measured within 1 mm. of the average length.

Egg (Fig. 2)

The egg is whitish when first laid, becoming yellowish with development of the embryo. It generally is flattened, oval in outline, fragile, and has a soft chorion. The average length is approximately 1 mm., the width being ⅔ mm. Eggs are deposited under bark scales bordering on cracks and crevices in the bark. Although eggs may be laid singly they more often occur in groups of two to eight; the largest group observed contained 13 eggs. Dissection of 29 females revealed from 7 to 242 eggs in various stages of development. The average was 122 eggs.

Incipient Stage (Fig. 3)

The small larvae of *M. californica* mine in the cambium region of green trees and stimulate the development of abnormal tissues in the sapwood and phloem. This phase of larval growth, which may last for several years, has been called the *incipient stage* since it represents a distinct stage preceding later stages of larval growth and successful development to adult. It usually represents an extended period of slow or practically arrested development that often terminates in death of the individual larva or of partial or entire broods.

Incipient stage larvae range in size from less than 1 mm. to 5 or 6 mm. in length. Small larvae, recently emerged from eggs, are difficult to see even after they have mined inward through the intervening bark and phloem zones to reach the cambial region. The whitish, nearly transparent, body scarcely is visible against the similarly coloured background of phloem and sapwood tissues. Sclerotized mouth parts and the fine brownish line of injured tissues behind the larvae are useful in determining their location. In very young incipient stage larvae the first thoracic segment is not noticeably wider than the following segments. During the period spent in the incipient stage the typical club-shaped form of *Melanophila* larvae develops.

Fast-growing Larval Stage (Fig. 4)

The significant contrast between the incipient and fast-growing stages lies in the relatively inconspicuous feeding and slow growth of the former as compared with the voracious feeding and rapid growth of the latter. Initiation of the fast-growing stage is characterized by a sudden widening of the incipient mine (Fig. 7) and the formation of winding galleries 10 to 15 mm. in width in the phloem. The frass in such galleries is usually packed in crescent shaped concentric units (Fig. 9).

The body length of fast-growing larvae ranges from 5 to 6 mm. for larvae that have recently entered the stage to 25 or 26 mm. for larvae that are ready to enter the prepupal stage.

Prepupal Stage (Figs. 5 and 6)

Towards the end of the fast-growing stage the larva mines outward into the cork-like layers of bark and constructs a pupal cell. In thin-barked trees the larva may construct this pupal cell in the sapwood. During the following prepupal stage the larva lies inactive in the pupal cell with its posterior abdominal segments bent forward to lie beside the thoracic segments in a U-shaped position. The prepupal larvae is relatively short, plump, and creamier in colour as compared with the fast-growing larva.

Pupal Stage (Fig. 6)

The pupa is of the exarate type with legs and wing pads folded against the body. In a majority of cases the pupa lies with its head directed toward the outer bark surface but individuals have been frequently observed in other positions. The average length of a series of pupae was about 9 mm. and the widest part of the body, in the abdominal region, was about 4 mm.

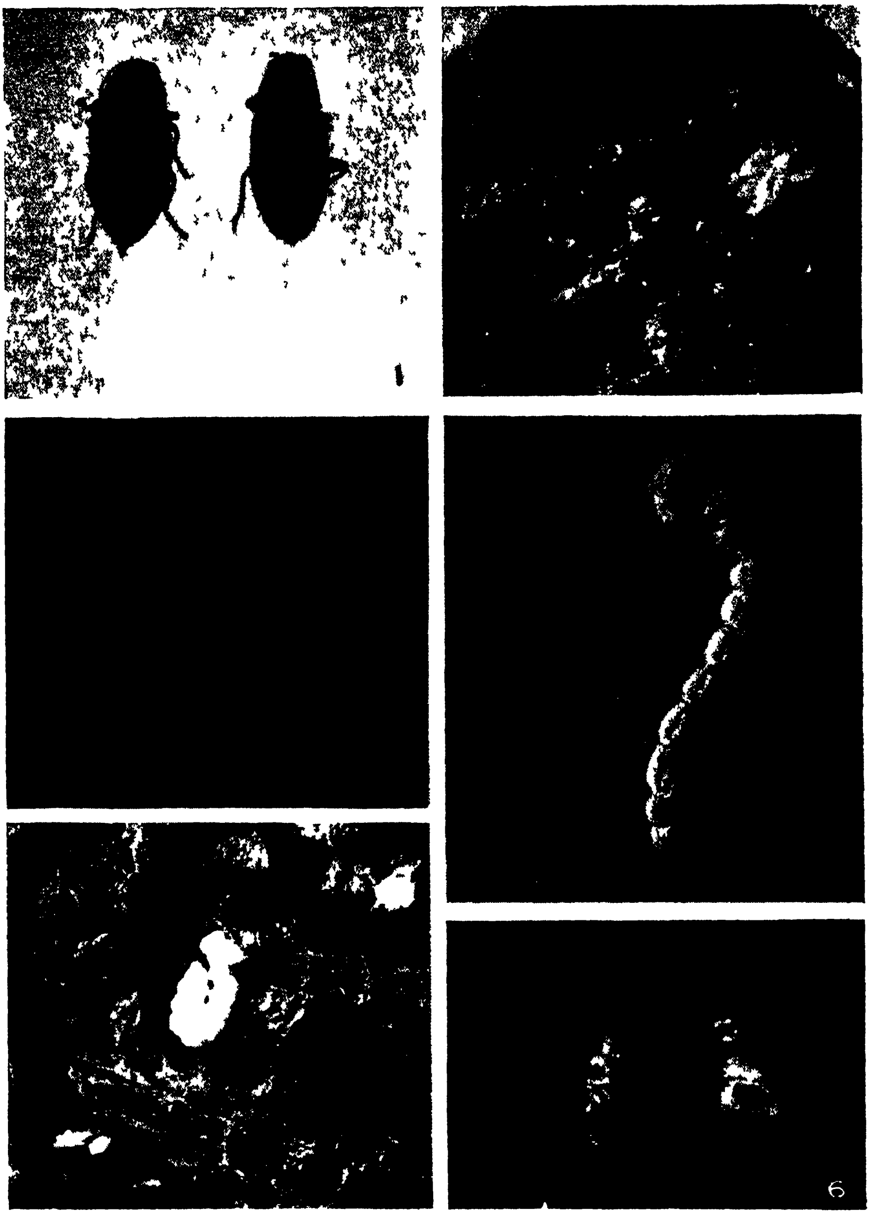
General Life History

Disregarding minor variations that are known to exist, the life history of a given brood of *M. californica* in northeastern California proceeds about as follows.

Eggs are laid under bark scales of host trees from June through August. They hatch in from 9 to 19 days, the average length of incubation being 13.3 days. After eclosion the young larvae tunnel diagonally through the intervening bark and phloem tissues to the cambium region where they remain for an indefinite period of time feeding on the living tissues of the tree and growing very slowly. Many individual larvae never develop beyond this stage and die. Frequently death is due to 'pitching out' as a result of resistance on the part of the host tree.

In trees where further development of the brood occurs incipient stage larvae continue to feed in the cambium region until the inception of the fast-growing larval stage, which appears to be dependent on host conditions that are as yet little understood. Usually that stage occurs during June to September one, two, or three years after the eggs were laid. There is some evidence to suggest that further development may occur after as many as four years in the incipient larval stage. Ordinarily fast-growing larvae have mined out into the bark, formed a pupal cell, and reached the prepupal condition before the advent of cold weather in late September or early October. Although the prepupal stage is ordinarily reached in the fall of the year, prepupae have been observed as early as the latter part of July. The following winter is spent in the prepupal stage; pupation begins in May but some larvae may pupate as late as July. Adults start emerging in May and the peak of emergence usually occurs early in July.

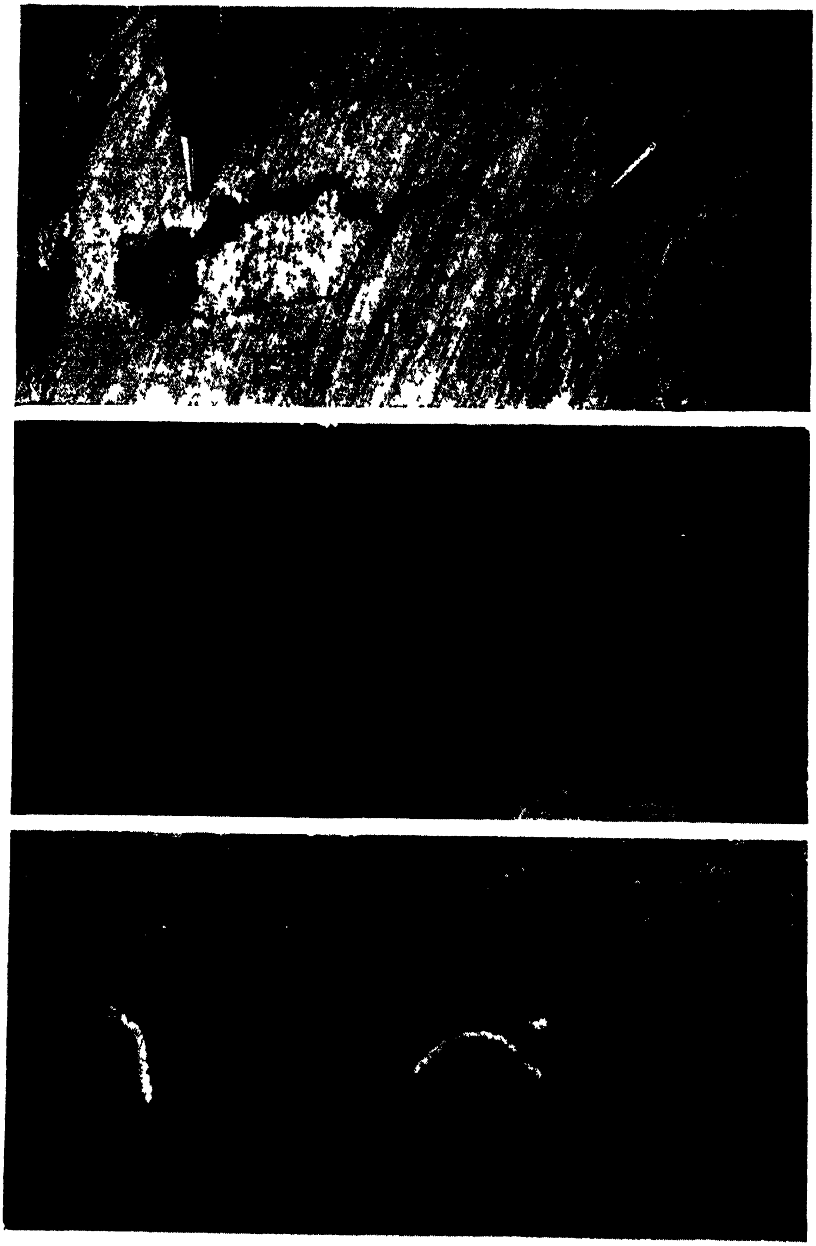
PLATE I



FIGS 1-6 *Developmental stages of Melanophila californica* Van Dyke

FIG 1 *Adult Approx* × *3*
FIG 2 *Cluster of eggs* × *10*
FIG 3 *Incipient larva and galleries in sapwood and phloem* × *2*
FIG 4 *Fast growing larva* × *2*
FIG 5 *Prepupal larva in characteristic position in bark* × *2*
FIG 6. *Pupa and prepupal larva in bark cells* × *2*

PLATE II



FIGS. 7-9 *Larval activity.*

FIG. 7. *Initiation of fast-growing stage Pencil on right points to start of incipient gallery Pencil on left points to fast-growing larva and its gallery Natural size*

FIG. 8. *Patches of deteriorating phloem covering fast growing larvae similar to the one shown in Fig 7. One-half natural size*

FIG 9 *Half grown fast-growing larvae showing utilization of deteriorating phloem tissue and larval galleries. Approx. natural size.*

Thus the life history of the species requires two to four, and possibly five years to complete. The general life history is shown diagrammatically in Fig. 10.

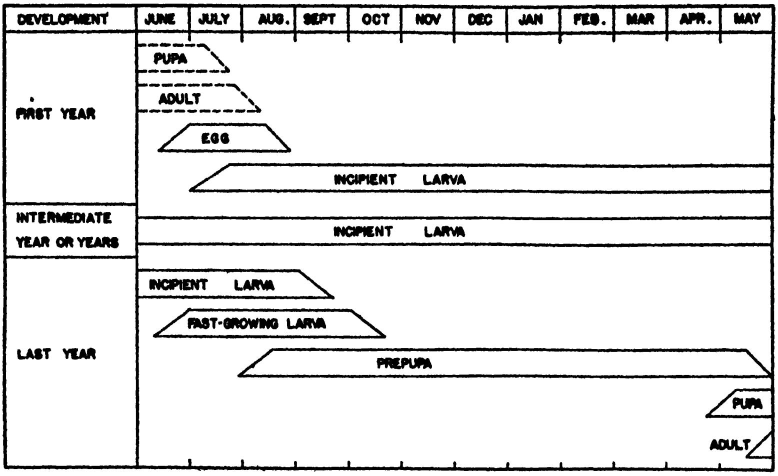


FIG. 10. *General progress of development of a single brood of M. californica.*

Variations in the Life History

Seasonal differences affect the rate of development of single generations of flatheaded borer broods and cause minor variations in the general life history. However, the greatest source of variation results from the actions of factors involved in determining when the change from the incipient to the fast-growing stage takes place. Those factors, the exact nature of which can only be surmised at this time, appear to determine the length of period spent in the incipient larval stage, the length of time necessary to complete the life cycle of individuals and of given generations, and the number of generations represented in the populations developing in the trees.

For some of the information contained in the following paragraphs the writer has drawn on a report by Johnson (8) that dealt with *atypical* broods and host materials.

Under certain conditions the life cycle may be completed in but one year. A few trees have been encountered in which it seems certain that a part of the population emerged during the summer following the attack (Johnson (8)). J. C. Evenden, in correspondence, reported a one year life cycle in felled poles in Idaho. In California the one year life cycle is of exceptional occurrence. Observations indicate that, in standing trees, it results from attacks made at or just before the time when conditions in the host tree become suitable for initiation of the fast-growing larval stage. Ordinarily such a condition occurs in the tree prior to the peak of adult emergence and by the time adults are ovipositing this material is no longer attractive as the result of contamination by other insect species.

In a majority of the infested trees that have been examined, three or four years have been required to complete the life cycle of individuals and of separate generations. A few individuals may, as has already been indicated, complete the life cycle in five years, which appears to be the maximum period that may be spent in developing from egg to adult. *Living incipient stage larvae have been found at the end of galleries that have been formed over a four year period* as indicated by annual rings overlying the galleries. Galleries formed over a similar four year period have been associated with broods that continued their development and emerged as adults one year after becoming fast-growing larvae.

Populations ordinarily overwinter chiefly in the incipient or the prepupal stage. However, occasionally the initiation of the fast-growing stage late in the fall season results in a relatively small proportion of the total population in the region spending the winter in that stage. In general these individuals are more susceptible to low winter temperatures than are incipients or prepupae. Ordinarily they do not continue their development in a normal manner during the following spring and most of them die before reaching the prepupal stage. A few observations have been recorded of larvae that overwintered in the fast-growing stage and subsequently completed their development successfully.

The prepupal stage is usually an overwintering phase. Exceptionally, this stage may be reached early in the spring, with pupation occurring during June or July of the same year. At the other extreme nearly a year may be spent in this stage by individuals that have been observed as prepupal larvae early in July and that did not emerge as adults until the following summer.

Numerous rearing records indicate that in practically all cases all the emergence from pieces of bark occurs during a single season. Examination of bark samples at the end of the emergence period did not indicate the presence of any diapause brood. In a few cases where *atypical* life cycle or periods of development were involved emergence from the same pieces of bark has occurred during two seasons. However, split emergence periods appear to be of infrequent occurrence.

Development of Populations in Standing Trees

In most incipient infested trees the larval populations present are representatives of more than one generation, having resulted from attacks of more than a single season. When the life cycle of these broods continues beyond the incipient stage and is successfully completed, adults resulting from the succession of attacks emerge during a single season. Thus there commonly occurs a considerable variation in the length of life cycle of the individuals and generations of the mixed-age broods that develop successfully to the adult stage in a single tree. This results in a somewhat complicated developmental history for the aggregate population that is living and developing in the cambium region of the infested trees of a stand. A reconstruction of a representative, though not necessarily average, developmental history of such a population is shown in Fig. 11. The explanation of this figure follows. From the time of first attack until approximately the time of initiation of the fast-growing larval period the host tree is living and usually forming annual growth rings. During the remainder of the developmental period for the flatheaded population the host material, which may be an entire tree or but a portion of a tree, is in first a dying, then a dead condition. Such feeding as takes place after initiation of the fast-growing stage is associated with the disintegration of host tissues.

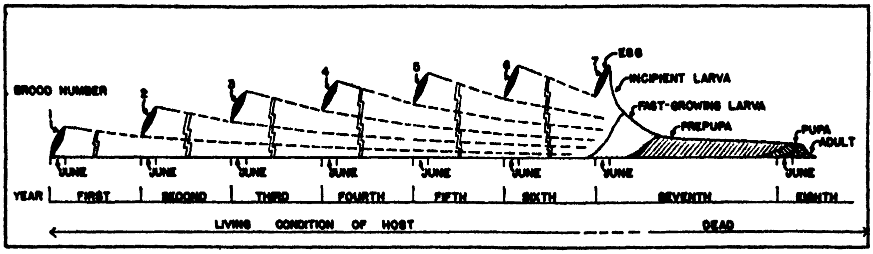


FIG. 11. *Infestation history of M. californica in standing trees.*

Successive attacks may be made in individual trees over relatively long periods of time. A detailed examination of cross sections from 35 flatheaded infested trees indicated that more than 55% of that sample had been recipient of successive attacks by incipient populations for between four and seven years. The range for the sample was from 1 to 13 years and the average between five and six years. On the basis of this information a six year period of attack has been used in Fig. 11. A seventh attack represents that of the season in which the fast-growing stage is initiated. The latter usually occurs but would not be evidenced by incipient scars in the sapwood. A year is usually required for the development from the end of the incipient stage to emergence. Thus seven and part of an eighth year may be involved in the complete developmental history of the flatheaded borer population.

Counts on approximately 130 sq. ft. of sapwood surface of infested trees showed an incipient stage larval population averaging 11.7 living larvae per square foot. A maximum population of nearly 35 living incipient larvae per square foot has been recorded. In many cases these larvae represent four seasons of attack, a point illustrated by the following table resulting from a limited series of counts made of living larvae existing in incipient infested trees during the 1937 season.

In the foregoing samples larvae resulting from the latest attacks are most abundant and those resulting from the oldest attack are least numerous. In addition to living larvae numerous scars that are not associated with living larvae occur. Often the dates of origin can be determined by cross-dating their location with the annual rings in the tree. These scars when added to the number of living larvae originating in the same year of attack provide a basis for determining the mortality of incipient larvae. The annual mortality

TABLE I

PROPORTIONS OF INCIPIENT POPULATIONS RESULTING FROM SUCCESSIVE SEASONS OF ATTACK

Sample No.	Year of origin of living incipient stage larvae							
	1934		1935		1936		1937	
	No.	% Total	No.	% Total	No.	% Total	No.	% Total
1	0	0	3	7	9	21	30	76
2	9	33	6	22	1	4	11	31
3	0	0	12	25	15	31	21	44
Total	9	8	21	18	25	21	62	53

was found to be in the neighbourhood of 30%, regardless of the age of the larvae. That point is brought out in the following table giving an example of the mortality of a single season.

TABLE II

MORTALITY OF INCIPIENT STAGE LARVAE, SUMMER, 1937

Sample No.	Year of origin of brood								
	1933			1936			1937		
	No. larvae		% Mortality	No. larvae		% Mortality	No. larvae		% Mortality
	Alive	Dead		Alive	Dead		Alive	Dead	
1	3	3	50	9	4	30	30	1	3
2	6	3	33	1	3	75	11	4	27
3	12	3	20	15	6	29	21	20	49
Total	21	9	30	25	13	34	62	25	29

In the schematic diagram of Fig. 11 the part individuals of each year of origin play in development beyond the incipient stage has been illustrated on the basis of the foregoing data. If six successive years of attack occur in a given tree it is likely that no individuals resulting from attack periods 1 and 2 will survive until the start of the seventh year. Approximately one-third of the living larvae of each year of origin die each year. For that reason Brood 3 will have very few representatives living in the seventh year of attack.

This drain on the populations of a given year of attack has been indicated in Fig. 11 by the slope of the solid top lines and their broken extensions. The total population present in the tree at any given time is represented by the vertical distance above the base line of the diagram. Broods 4, 5, and 6 are the chief ones contributing to the population that enters and continues development through and beyond the fast-growing larval stage. Brood 7, which results from attacks at the time the remainder of the population is entering the fast-growing stage, is the most abundant single brood represented in the population active in the tree at that time. Some individuals of Brood 7 may progress to the fast-growing stage and compete successfully with older larvae in subsequent development, but for the most part mortality in that portion of the population is usually heavy and few, if any, individuals of Brood 7 would ordinarily complete development and emerge. Thus a large portion of the mortality in the fast-growing stage, which results in a population reduction from 11.7 incipient stage larvae per square foot to about six prepupal larvae per square foot, appears to occur among individuals of the brood resulting from the seventh period of attack.

The remainder of the diagram illustrates the usual timing and sequence of events in development through the prepupal and pupal to the adult stage.

In nature the complete infestation history of individual trees is usually more complicated than is shown in this reconstructed infestation history of the California flatheaded borer. Partial infestations, such as top or strip kills, may occur, involving overlapping infestation histories. Often the initial flatheaded borer infestation in parts of a tree is followed by supplementary attacks by other species of forest insects. In this manner single or multiple infestation histories in different portions of a tree bole and the larger limbs may result and there is commonly a succession of flatheaded borer or other forest insect infestations.

A typical succession that has been commonly recognized in east-side ponderosa pine stands is illustrated in Plate III. Figs. 12*a* and 13*a* are two views of the same tree, taken over a year apart, and Figs. 12*b* and 13*b* are two diagrams illustrating the composition of infestations in the tree at the times the photographs were taken. The first photograph was taken on June 21, 1935, and the second on August 30, 1936.

Sapwood scars caused by successive infestations by incipient flatheaded borer larval broods were found along the entire bole length of this tree. Different series of incipient flatheaded borer broods reached the fast-growing stage in 1934 (in top 30 feet of bole length), and in 1935 (between 35 and 60 ft. from the base). These different series emerged as adults in 1935 and 1936 and have been labelled *FH*-1934 and *FH*-1935 in the diagrams to distinguish between them.

Supplementary attacks by adults of the 1935-1936 overwintering western pine beetle broods took place early in the spring of 1936 (*DB*-1936). The result was the pure western pine beetle infestation found from 18 to 35 ft. up the bole and the mixed flatheaded borer–western pine beetle infestation extending from 35 to 60 ft.

Another attack period, this time by western pine beetle adults of the first 1936 seasonal generation and by mountain pine beetle adults (*DM*-1936), took place some time in July, 1936. These two attacks resulted in a mixed infestation by the two species in the basal 17 ft. of the bole length.

The flatheaded borer attacks probably took place over a period of years. If we consider only that this series of successive attacks successfully completed development to adult stage in two different periods of time it is evident that four separate infestations by three species of forest insects are shown by this example.

Habits

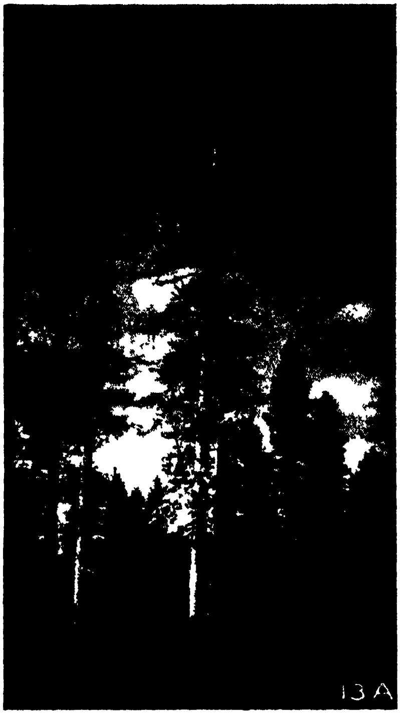
Adult

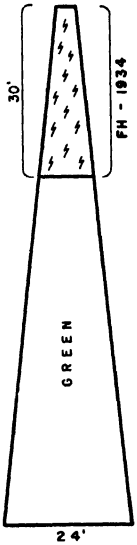
Adults are found in pupal cells in the outer bark of infested trees from April until late in July. The two sexes emerge at about the same time and in approximately equal numbers. The average emergence was 3 6 beetles per square foot of bark surface, from approximately 2700 sq. ft. of bark representing a number of samples over several seasons. The beetles are active on warm (70° F. and above) sunny days, but are rarely seen in flight. They can occasionally be collected from pine foliage and are attracted, often in large numbers, to windfalls, slash, and other freshly felled host material. They have been observed crawling over the bark of trees supporting heavy incipient infestations. Temperature, rather than light, appears to be the factor controlling activity. The beetles are inactive at night, in the early morning, and in the late afternoon However, they are positively phototropic, exhibiting a preference for sunlight areas on logs and trees and moving to light-admitting apertures in darkened cages.

For a period of about 10 days following emergence, the adults spend their time flying about and feeding on pine foliage. This feeding appears to be necessary for sexual maturity. In cages neither copulation nor oviposition was observed unless fresh foliage was furnished as food for the caged populations. The effect of adult feeding under natural forest conditions is not known, although feeding injury was found on from 54 to 100% of the needles of 181 green twigs that were taken as samples from eight incipient infested trees. When relatively large populations of adults are confined in cages placed over small trees the feeding injury has been severe This injury resulted in the death of many needles, a general thinning of the foliage and a consequent reduction of the physiologically active needle complement. The extent of injury to caged pole-size ponderosa pines is shown in Table III.

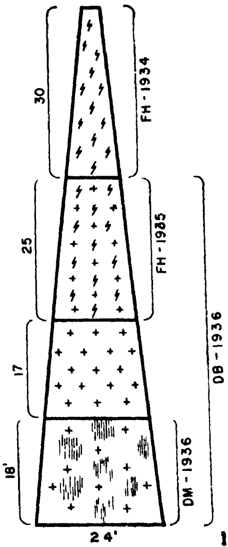
Under caged conditions the beetles preferred needles of the current or previous season although some feeding occurred on needles of all ages. Individual needles on which adults have fed exhibit a series of irregular serrations along the edge. Ordinarily the edges of the serrations are not clean cut. When the injury extends well into the centre the needle usually dies beyond the point of attack, or the entire needle may be killed.

There is considerable variation in length of adult life. Under cage conditions, with fresh pine foliage supplied as food, one adult lived for 62 days after emergence. The average length of life of 344 adults under those conditions was 13 days. Without food the average age at death was but four days, with a maximum of 15 days.





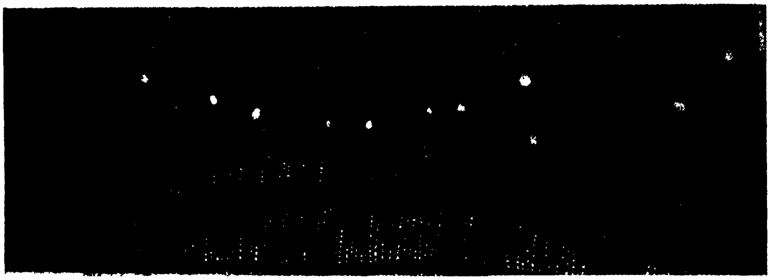
12B

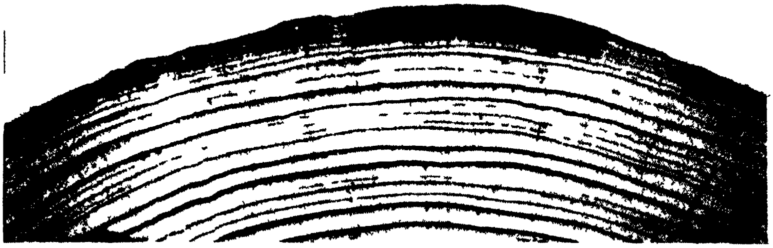


13B

FIGS. 12 and 13. *Example of a common sequence of successful infestations in east-side ponderosa pine trees.*

FIG. 12*a*. *Picture of tree on June 21, 1935.*
FIG. 12*b*. *Diagram of infestation in tree on June 21, 1935.*
FIG. 13*a*. *Picture of tree on Aug. 30, 1936.*
FIG. 13*b*. *Diagram of infestation in tree on Aug. 30, 1936.*





FIGS 14 17 *Effect of incipient galleries on wood tissues*

FIG 14 *Cross section of incipient gallery overlaid by wood tissues showing resin infiltration and parenchymatous tissue* × *16*

FIG 15 *Cross section of old incipient gallery in sapwood showing growth disturbance and abnormal concentration of resin ducts* × *25*

FIG 16 *Pitch pocket resulting from incipient attack Natural size*

FIG. 17 *Old incipient gallery and pit showing effect on subsequent growth* × *2*

TABLE III

EXTENT OF INJURY CAUSED BY FEEDING OF ADULTS ON NEEDLES OF CAGED PONDEROSA PINE POLES

No adults in cage	Age of needles[1]					
	Current season		One year old		Two years old	
	Fascicles injured, %	Needles injured, %	Fascicles injured, %	Needles injured, %	Fascicles injured, %	Needles injured, %
214	61.6	39.6	69.1	41.6	9.9	4 6
358	31.4	16.7	32.3	19 4	2.3	0.9
173	22.8	12.9	2.6	1.2	4.8	2.3

[1] *Ponderosa pine normally has a needle complement of three seasons while in Jeffrey pine the needles are retained for six to nine years.*

Oviposition

When ready to oviposit the adult crawls about over the bark and, when a suitable place is found, exerts the ovipositor to as much as two-thirds the length of the body. The eggs are placed either singly or in small groups on bark scales bordering on the crevices between bark plates. The percentage hatch of a sample of 1597 eggs observed was low, averaging but 50.8%.

Incipient Stage

Hatching occurs through the side of the egg and the larva mines diagonally through the bark to the cambium region. There, until the larva attains the fast-growing stage or dies without successfully completing the incipient stage, it feeds on the tissues of the cambium region.

The feeding injury consists of the excavation of a shallow mine in the cambium and immediately adjacent innermost phloem and outermost wood tissues. Technically the open mine or gallery of the incipient stage larva extends but a short distance behind the larva. Cellular proliferation heals the gallery relatively soon after the injury occurs. The gallery may extend ahead of the larva for about a quarter of an inch. The larva itself lies in an irregularly shaped shallow cavity that may be lobed as a result of the larva starting to mine in more than one direction. Frass fills the cavity immediately behind the larva.

The healed over mines have been named incipient galleries and are the result of typical reaction by the trees to wounding. These galleries vary in length from less than an inch to as much as five or six inches and represent the extent of feeding by individual incipient-stage larvae during the period of time spent in that stage. In the sapwood the reactions of the tree form a ridge of tissue (Figs. 3, 7, 14) infiltrated with pitch. Proliferation of parenchymatous tissue, dislocation of wood tracheids, the production of an abnormal number of resin ducts (Figs. 14 and 15), and a lack of continuity of medullary rays through the wound tissue are usually the results of the feeding injury. Usually the growth of the season following the injury is normal but the annual ring over the gallery often is wider or narrower than in adjacent uninjured areas (Fig. 15). Sometimes growth of a normal character or amount does not occur for several years.

When the larva dies without completing the incipient stage the point in the tree at which death of the larva occurs usually is marked in one of three ways. If the tree actively resists the attacks a flow of resin may overcome and imbed the larva. Subsequent healing leaves a pitch blister as a defect in the wood (Fig. 16). In the absence of very active resistance on the part of the tree the point of death may be marked by a pitchy fleck that subsequently becomes covered with normal wood tissue. Sometimes a pit is formed in association with the pitch fleck and distortion of annual rings of subsequent growth may involve the development of many seasons (Fig. 17).

Phloem disturbance is evidenced in brown, corky, injured cells whose pattern is a counterpart of the sapwood incipient gallery.

Fast-growing Larval Stage

The factors resulting in initiation of the fast-growing stage are unknown, but it appears that cessation of growth and probably death of the tree or infested portion of the tree occurs at about this time. Change in the character of the larval mine and in the rate of larval feeding and growth are unmistakable evidence of the transition from incipient to fast-growing stage. The mine is abruptly widened (Fig. 7) beyond the point where the incipient gallery ends. Frass is packed in crescent shaped units instead of being scattered loosely. Larvae feed voraciously on phloem tissue and increase in size very rapidly. When the outer bark is carefully removed from the phloem at the time of initiation of the fast-growing stage, browned phloem patches can be observed (Fig. 8). Living larvae are found under these patches. The mines are extended rapidly and feeding results in a relatively high utilization of deteriorating phloem tissue (Fig. 9).

Brood Mortality

Natural Enemies

Little is known concerning the natural enemies of *M. californica*. Relatively few instances of parasitism and predatism have been encountered. In the course of studies on the egg stage a small hymenopteran was discovered parasitizing eggs immediately after they were placed. The parasite was determined by A. B. Gahan as a new species of an encyrtid of a genus not previously known to attack buprestid eggs. The percentage of parasitism was low. In a study made under conditions that did not interfere with the normal activity of the parasite, 2.9% of 1178 eggs were attacked.

A few parasitic Hymenoptera have emerged from bark samples containing flatheaded borer broods but no host–parasite relationship was specifically established. The numbers involved were so small as to indicate no appreciable effectiveness of parasites of the California flatheaded borer. The protected position of borer broods under the bark naturally limits the opportunity for parasitism to occur.

Clerid and ostomatid predators, chiefly *Thanasimus lecontei* (Wolc.) and *Temnochila virescens* (Fab.) have been observed attacking flatheaded borer larvae in infested trees. Larval stages of these predators commonly are found under the bark of infested trees after attacks by bark beetles have provided a means of access to the inner bark region.

Woodpeckers, chiefly the arctic three-toed woodpecker (*Picoides articus*) commonly destroy a considerable proportion of the overwintering prepupal larvae. In 1938 a count on infested bark taken from standing trees showed 23% of the original overwintering prepupal brood had been destroyed by woodpeckers.

Competition

Under some conditions competition for food appears to be an important factor in reducing flatheaded borer populations. Instances have been observed in which other forest insects, particularly the engraver beetle (*Ips oregoni*) and the western pine beetle (*Dendroctonus brevicomis*), have overwhelmed flatheaded borer broods. These insects, attacking soon after the flatheaded broods have reached the fast-growing stage, develop more rapidly than the flatheaded larvae. Most of the available food is consumed before the latter have completed their development and a large portion of the brood dies. There appears to be a considerable variation in the mortality resulting from this competition. This variation largely stems from the timing of attacks relative to the stage of development reached by the flatheaded borer broods. The earlier these attacks occur after the fast-growing stage has been reached the greater is the resulting mortality in the fast-growing larval population. If the attacks occur when the fast-growing stage is well advanced the flatheaded larvae are able to withstand competition and many of them continue their development to a successful emergence. Success in withstanding competition depends, too, on the species of competitor and its rate of development. For example, the Jeffrey pine beetle (*D. jeffreyi*) in Jeffrey pine develops more slowly than does the western pine beetle in ponderosa pine. Flatheaded borer broods compete more successfully with the Jeffrey pine beetle.

Temperature

The normal overwintering stages of the flatheaded borer usually withstand minimum temperatures even though such temperatures are low enough to cause considerable mortality of broods of other forest insect species overwintering in the same infested trees. The fast-growing stage seems to be most susceptible to the effects of low temperature. In laboratory tests exposure for two hours to a temperature of −15° F. resulted in mortality as high as 62.5%.

Air and the resulting bark temperatures during the spring and summer ordinarily do not constitute a mortality factor. However, some effects are evident when temperatures of 50° F. or less retard the pupation of overwintering prepupal larvae during the spring season. In broods that were experimentally exposed to temperatures between 50° and 55° F. for periods ranging from 64 to 93 days emergence was reduced 24 to 61% as compared with brood exposed to normal temperatures.

Nutritional Factors as Relating to Mortality and Host Materials

Experimental studies to determine in what manner the character and qualities of available host material relate to success of development of flatheaded larvae broods were not undertaken. It is already apparent that the host–insect relationship is an important one in determining if and when broods of the California flatheaded borer are to develop beyond the incipient stage and the degree of success of development beyond that stage. Presumably this relationship would be largely concerned with nutritional factors.

The rate of mortality in the incipient stage is high. Following attack, even in the least resistant trees, a period of feeding by incipient larvae is the rule. It has already been indicated (Table II) that when the incipient stage lasts through more than one season the annual mortality is in the order of 30%. Quite probably the terms 'host resistance' and 'nutrition' are synonymous as relates to the success of incipient larvae.

Apparently host resistance serves to hold back growth and development of the incipient stage larvae. The transition from incipient to fast-growing stage appears to involve changes either in the character of the food available or in the ability of the larvae to utilize that food. The success of subsequent development of broods in the fast-growing stage also appears to be conditioned by the physical and chemical nature of the food available. These assumptions have considerable basis if interpretations of the brood and emergence data for different types of host materials are possible at this time.

Although the tissues of the cambium region of living trees appear to be the normal food of incipient larvae, the relatively high rate of mortality and the apparent inability of the larvae to develop beyond that stage until some change takes place in the host tree suggests an imperfect adaptation of the insect to conditions in the living tree. On the other hand, the distinct preference for living trees for attack, the ordinarily poor development and high rate of mortality in fresh slash, and the inability to attack successfully in the uninfested cambium region of green slash suggests a somewhat similar imperfect adaptation to slash host material. In its biology this species is thus closer to the fir flatheaded borer (*Melanophila drummondi* Kby.), which Hopkins (7) considered to be an important enemy of Douglas fir or to the bronze birch borer (*A. anxius*, Balch and Prebble, 2) than to the hemlock borer (*M. fulvoguttata*), which Secrest *et al.* (12) consider to be secondary and relatively unimportant in the dying of the eastern hemlock. The pine flatheaded borer (*M. gentilis*) is another example of the latter type.

It is emphasized again that there is an evident need for experimental studies to determine how the character and qualities of the available host material affect the development of both incipient and fast-growing larvae and what are the normal nutritional requirements of the species if there is to be an adequate factual basis for interpreting the role of the species in the complex western pine beetle problem.

Host Materials

Broods of *M. californica* develop to maturity in the boles and larger limbs of standing trees, windthrown or otherwise injured trees, and in cull logs, tops, and the larger limbs left as slash following logging operations. It is emphasized that with rare exceptions, regardless of type of host material from which the emergence occurs, healed incipient galleries have been found thus indicating that attack occurred in the *living tree.* Successful attacks occur on fresh phloem but deteriorating or browned phloem is either not attractive or not suited to the development of the early larval stages.

Emergence of adults gives the most readily available indication of the suitability of various types of host material and makes possible a comparison of the relative success of *established* incipient broods in the various types of host material. In all cases bark for emergence samples was removed from trees or logs only after the brood had reached the prepupal or pupal stage of development.

Standing Trees

The attractiveness of standing trees as normal host material is indicated by the occurrence of incipient populations of medium to heavy intensity in 29% of a random sample of 395 ponderosa pines. The condition of the host tree as a factor in selection for attack, or in success of establishment of incipients, is indicated by the higher incidence of incipient broods in so-called high-risk green trees (Salman and Bongberg, 11). However, the attractiveness of certain individuals or types of trees does not necessarily indicate their suitability for development of the broods to maturity.

Emergence from numerous samples of bark varied from zero to a maximum of 14.6 beetles per square foot of bark surface. This variation was exhibited in records of different individual trees or even different parts of a single tree. The average figure for several seasons are given in Table IV.

TABLE IV

ADULT EMERGENCE FROM BARK TAKEN FROM NATURALLY INFESTED STANDING TREES

Season of record	Sq. ft. in bark sample	Emergence, no. beetles per sq. ft.
1936	319	3.6
1937	305	2.9
1938	500	4.2
1939-1940	71	5.7
All records	1195	3.8

Strip-killed or top-killed material usually gave a less than average emergence. The same was true of bark from the lower bole region of infested trees. In 1936 a 60 sq. ft. bark sample, taken from the basal 10 ft. of five infested trees, gave an average emergence of only 1.2 beetles per square foot and emergence from all five samples was less than the seasonal average of 3.6. Similar poor emergence from the basal bole region has been noted for other insects attacking ponderosa pine.

Slash, Cull Logs, and Tops

Slash may result from logging operations during any month of the year. Such material may be uninfested, may contain incipient broods, or occasionally broods in infested but still green trees have reached the early fast-growing stage. Up to the time the investigations reported in this paper were completed it had not been possible to rear *M. californica* adults from slash or other cut material that was uninfested at the time of cutting. Oviposition and light early incipient infestations have been forced on previously uninfested logs in cage experiments but in no instance was the brood carried successfully to maturity. However, J. C. Evenden of the Coeur d'Alene laboratory of the Bureau of Entomology and Plant Quarantine has reported in correspondence that *M. californica* adults have been secured from previously uninfested ponderosa pine slash. Thus it may be concluded that the establishment and development of flatheaded borer broods can occur in slash after it has been laid down, but in northeastern California this type of infestation is not known to occur.

In slash from incipient infested trees the fast-growing stage is initiated soon after cutting when cutting occurs during the summer months. The stage of development at the time the slash is laid down appears to be important in determining the degree of success of broods in subsequent development to maturity, as is illustrated in the following table.

TABLE V

EMERGENCE FROM SLASH

Year	Cut with brood in incipient stage		Cut with brood in fast-growing stage	
	Sq. ft. bark in samples	Emergence per sq. ft.	Sq. ft. bark in samples	Emergence per sq. ft.
1936	156	3.3	174	2.1
1937	55	.5	—	—
1939-1940	22	4.6	143	6.7
All samples	233	2.8	317	4.2

Apparently slash created when the brood is in the fast-growing stage is more satisfactory host medium for subsequent development than is slash created when the brood is in the incipient stage. Analysis of individual records indicates that the average emergence figures given in the preceding table are not entirely representative and that in general slash is not a particularly favourable type of host material.

On the whole, experience indicates that the cutting of trees containing incipient stage larva will initiate the fast-growing stage. Subsequent development appears to be variable, generally being abnormal and relatively unsuccessful. It may be assumed that much of the variation in development of broods in slash is associated with differences in nutritional factors. This assumption cannot be substantiated until more is known of the nutritional requirements of flatheaded larvae and of the physico-chemical changes that take place in host material after it is cut.

Windfalls

Records from 112 sq. ft. of bark samples taken from incipient infested windthrown trees gave an average emergence of only 1.7 beetles per sq. ft. It would appear that this type of host material also is an unsatisfactory medium for the development of those broods to maturity. As in the case of slash, competition by other insect species is one of the important factors regulating development of established incipient broods.

Injured Trees

Many trees that become infested by forest insects, including flatheaded borers, are fire-scarred or otherwise injured at the base. The question of the effect of these and other injuries has frequently been raised. In 1939 four incipient infested pole-size ponderosa pines were girdled by a saw kerf to test the effect of girdling on subsequent development of the broods. Two poles were girdled by twisting a heavy wire tightly around the base. The effect of that constriction would not be evident for several years. Two trees of the first series died in 1940. In one of these woodpeckers removed the major portion of an apparently heavy prepupal brood before samples could be taken. Adults of *M. californica* were secured from the bark of another dead tree in 1941. This tree had been girdled at a height of 6 ft. Above the girdle there was an emergence of 14.8 beetles per square foot. Below the girdle incipient development continued for a time and the fast-growing stage was reached but apparently no individuals reached the prepupal stage. In a pole that was practically girdled at the base by fire about a year before it succumbed to the flatheaded borer, emergence averaged 8.5 beetles per square foot of bark surface. No definite conclusions can be drawn from information supplied by two trees but the very successful brood development above injuries that disrupted conduction in the phloem may be significant.

Effect of Insect on the Host

At the present time no attempt is being made to define the role of the California flatheaded borer as a factor in the western pine beetle problem. There remains to be done a considerable amount of investigation before the cause and effect phases are clear.

The occurrence and greater success of the borer in weaker or decadent types of trees has been noted. Thus a preceding factor, climatic or other, may relate to the incidence and success of attack. Regardless of the existence of the predisposing factor the habits of the species are such that a detrimental effect on the host may result. The borer may be one factor in bringing about the death of the tree. Commonly the western pine beetle is the last factor in the chain of phenomena leading to death, but some trees may die with the flatheaded borer being the only insect present.

The possible effects of defoliation by adult feeding are probably negligible. However, the occurrence of abnormal wood tissue as the result of incipient larval activity may have a significant effect. Any attempt at explanation of the relation of incipient galleries to the host tree must consider the effect of such galleries on conduction in the wood. Although admittedly a single gallery in itself would be insignificant, the sum total of galleries may present a considerable amount of abnormal tissue in the line of conduction.

Initial experiments on the relation of incipient galleries to conduction have indicated that some interference with normal conduction occurs. These experiments consisted of injecting a stain into small pieces of wood, logs, and entire trees and examining the conduction pattern in relation to the incipient galleries present. Conduction does not occur through the abnormal parenchymatous tissues that heal the larval mines. The possible physiological effects of this interference would be intensified according to the degree of infestation present in a tree. It is generally understood that the outer layers of wood are the most physiologically active. Thus a succession of incipient attacks may seriously reduce the conducting capacity of the bole.

When standing living trees were injected the stain failed to reach the top of trees that supported moderate to heavy incipient infestations. Since such trees had a poorer needle complement as compared with check trees in which the stain reached the top, the result is by no means entirely due to the presence of incipient galleries. It is of importance to note that in all cases the conduction stream broke up in the region of the bole where incipient galleries were present.

Although there is the evidence that incipient galleries interfere with normal physiological activity, considerably more study is necessary before the effect of this interference and the consequent role of the borer can be evaluated.

Summary

The frequent occurrence of the California flatheaded borer (*Melanophila californica* Van Dyke) in infestation of commercial pine stands of northeastern California and the lack of specific information concerning its life history and habits have warranted an investigation of the species as a step in determining its place and importance in the so-called western pine beetle problem. This insect is known to infest several species of pine, but in northeastern California is important chiefly because it is concerned in insect-caused losses in valuable mature stands of ponderosa and Jeffrey pines.

The eggs are laid in bark crevices on living trees from about mid-June through August. After hatching, the larvae bore through the phloem to the cambium region where they feed on living tissues for a period varying from a few months to a maximum of about four years. In general the majority of larvae spend one, two, or three years in the cambium region in what has been termed the incipient stage. Ultimately these larvae die if the tree does not succumb, or if the tree dies they pass to a fast-growing stage in which feeding is extensive and growth is rapid. Usually fast-growing larvae are found from mid-June to mid-October but may occur at any time of the year. The prepupal larval stage first appears in the outer bark late in July, but pupation does not occur until April and May of the following year. Adults are active from late in May until early in August.

In standing trees, infestations are complicated by the occurrence of successive attacks over a period of years. Larvae of different ages that result from these successive attacks live together in the cambium region. The situation is further complicated by supplementary attacks in flatheaded borer infested trees by several bark beetle species.

Standing trees are infested in significant numbers. The older, decadent, or unhealthy trees have a higher incidence of infestation but thrifty and vigorous trees are not immune to attack. Adults have also been reared from slash or cull logs from trees infested while living and from windthrown and injured trees.

More work remains to be done, particularly on the host–insect relations before it will be possible to definitely determine the role of this species.

Acknowledgments

The work reported herein was carried out between 1935 and 1940 in the field at Hackmore, Modoc County, Calif. and in the laboratory at Berkeley, Calif. From 1935 through 1938 the work was supervised by K. A. Salman assisted by J. W. Bongberg in 1935 and the writer in 1936, 1937, and 1938. In 1939 the writer was in charge of the work and in 1940, J. W. Johnson worked under the direction of K. A. Salman in completing a few unfinished phases of the project.

The contributions of R. F. Anderson, H. E. Burke, Jr., D. P. Furman, D. R. Rankin, and T. L. Thompson, who acted in capacity of field or laboratory assistants at various times, were of considerable importance. Grateful acknowledgment also is made to those former associates in the Division of Forest Insect Investigations of the Bureau of Entomology and Plant Quarantine who have aided the work by their criticisms and suggestions. In particular the writer is indebted to J. M. Miller, formerly in charge of the program of forest insect investigations at the Berkeley laboratory, for his advice and counsel, and to K. A. Salman, formerly Entomologist, under whose direction this study was conducted, for detailed assistance in revisions of this manuscript. Credit for photographs is due to K. A. Salman.

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THE PROVENTRICULUS OF *STAPHYLINUS BADIPES* LEC. (COLEOPTERA : STAPHYLINIDAE)[1]

BY W. W. JUDD[2]

Abstract

The proventriculus of the rove-beetle, *Staphylinus badipes* Lec., is described. It is similar in form to the proventriculi of beetles of the Staphylinidae and Silphidae, described by other authors, in that its sclerotized lining is arranged in eight longitudinal ridges alternating with eight rows of fine hairs.

Materials

Specimens of *Staphylinus badipes* Lec. were found beneath rocks and logs in damp spots in woods about Ottawa, Ont. on June 20, July 3, and Sept. 6, 1939. They were identified by Mr. W. J. Brown, Division of Systematic Entomology, Department of Agriculture, Ottawa.

Preparatory to making a whole mount of the sclerotized intima of a proventriculus, the digestive tract of a beetle was carefully removed from the body cavity. It was pinned out, under water, on a layer of wax in a Syracuse watch glass, and the proventriculus was slit longitudinally with a pair of fine eye scissors. The proventriculus was cut from the digestive tract and placed in a strong solution of potassium hydroxide, for a few hours, until the layers of muscle and other soft tissues had been dissolved. The hydroxide was then washed out with water, and the sclerotized intima was then washed with a series of alcohols of increasing strength. Then, after washing with xylol, the intima was mounted in Canada balsam.

Serial transverse sections of a proventriculus were made and stained with haemotoxylin and eosin.

Description

The proventriculus is a spindle-shaped structure and is about 2 mm. long.

The thin, sclerotized intima (I, Figs. 2, 3) is organized into eight longitudinal ridges. In the anterior portion of the proventriculus four of these ridges are larger than those alternating with them (Fig. 2); in the body of the proventriculus the ridges are of equal size (Fig. 3). In the anterior region the crests of the four large ridges are beset with small spines (Fig. 2); in the body of the proventriculus there are long fine hairs that arise from the intima at the sides of the ridges and between the ridges (CH, Figs. 1, 3). These hairs project into the lumen in eight separate clusters that meet at the centre of the lumen and tend to intermingle (Fig. 3). The flattened-out intima of

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[1] *Manuscript received January 15, 1947.*

Contribution from Department of Applied Biology, University of Western Ontario, London, Ont.

Part of a thesis presented in accordance with the regulations for the degree of Master of Arts of the University of Western Ontario, London, Ont.

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the proventriculus has the appearance shown in Fig. 1. The rows of hairs are arranged in pairs, and between these pairs of rows are smaller subsidiary rows of shorter hairs.

Beneath the intima is a layer of epithelial cells of irregular shape (EP, Figs. 2, 3, 4) and in the cavities of the longitudinal ridges are strands of longitudinal muscle (LM, Figs. 2, 3). Forming the outer layer of the proventriculus is the circular muscle (CM, Figs. 2, 3, 4). It is two to four strands thick except in the region of the oesophageal valve (Fig. 4) where it is four to six strands thick.

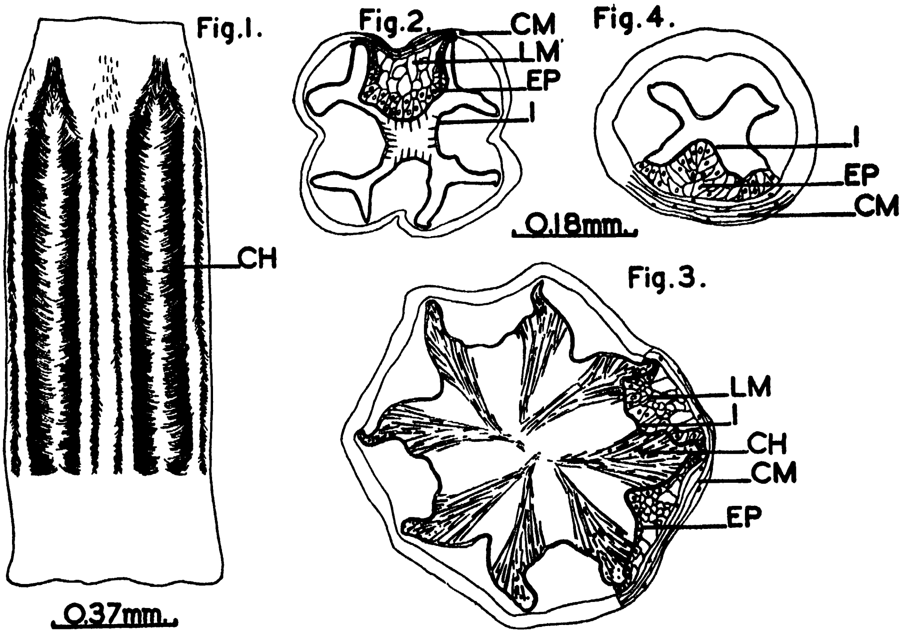

FIG. 1. *Part of the sclerotized intima of the proventriculus showing four of the eight rows of hairs.*

FIG. 2. *Transverse section of the anterior region of the proventriculus.*

FIG. 3. *Transverse section of the middle region of the proventriculus.*

FIG. 4. *Transverse section of the oesophageal valve.*

CH—coating of sclerotized hairs; CM—circular muscle; EP—epidermal layer; I—sclerotized intima; LM—longitudinal muscle.

In the region of the oesophageal valve (Fig. 4) the intima is arranged in a few irregular folds, and bears no coating of hairs.

Discussion

The proventriculus of the rove-beetle, *Creophilus villosus* Grav., is described by Talbot (4). Her description indicates that the proventriculus of *C. villosus* and *S. badipes* are similar. In both species the intima is organized in eight longitudinal ridges and the eight rows of fine hairs project into the lumen.

Heymons and von Lengerken, in a series of papers on the family Silphidae, described the proventriculi of the silphid beetles *Phosphuga atrata* L. (1), *Ablattaria laevigata* F. (2), and *Silpha carinata* L. (3). Their figures indicate that the proventriculus of beetles of this family is similar to that of the Staphylinidae. Eight longitudinal folds are evident, and they are beset with a coating of fine hairs projecting into the lumen.

Acknowledgment

The writer wishes to thank Professor J. D. Detwiler, University of Western Ontario, for his assistance and interest in this work.

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ON THE CHEMOTHERAPY OF CAECAL COCCIDIOSIS (*EIMERIA TENELLA*) OF CHICKENS

III. OBSERVATIONS ON SULPHONAMIDES IN THE INTESTINAL TRACT AND BLOOD[1]

By W. E. Swales[2] and G. H. Benham[3]

Abstract

Analyses of the contents of the caecal pouches and the small intestine have been made on chickens sacrificed four hours after dosing with sulphonamides. Sulphamerazine was found constantly in the caeca in small amounts even when the caeca were surgically detached from the rest of the intestinal tract, thus showing that the drug is excreted via the caeca to some extent. This property was found to be common also to sulphathiazole, sulphapyridine, and sulphanilamide. The importance of this finding lies in the fact that caecal coccidiosis of chicks is inhibited by sulphamerazine but not by the other three sulphonamides mentioned. It follows that the inhibitory effect is related to drug structure rather than to drug concentration.

The work by Horton-Smith and Taylor (4 and 5), Swales (12 and 13), Hawkins (3), Levine (6–9), Farr and Allen (2), and Ripsom and Herrick (11) on the use of sulphonamides in the control of acute coccidiosis of chickens raises a problem in regard to the mode of action of these drugs in inhibiting the parasite. The drugs that have been shown to have a marked coccidiostatic effect are sulphadiazine (2-sulphanilamidopyrimidine), sulphamerazine (2-sulphanilamido-4-methylpyrimidine), and sulphamezathine or, in the United States, sulphamethazine (2-sulphanilamido-4,6-dimethylpyrimidine). As these drugs are effective in controlling the disease even after haemorrhage from the intestinal tract has begun, it is evident that they inhibit the merozoites and thus prevent or suppress the repetition of schizogony. This effect was suggested for the sulphonamide-pyrimidine drugs by Swales (12) and independently for sulphadiazine by Ripsom and Herrick (11). It is also evident that other sulphonamides, notably sulphaguanidine and sulphathiazole, while not having a curative effect, do prevent acute coccidiosis caused by *Eimeria tenella* when they are given at the time of infection; thus it may be presumed that in the case of these drugs their action in inhibiting the parasite is confined to the sporozoites.

The ability of the sulphonamide-pyrimidine drugs to inhibit a parasite in the caecal pouches of chickens is more peculiar when the fact that these drugs have the characteristic of being rapidly absorbed from the upper digestive tract of animals is considered. Also, the caecal pouches of birds are not

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[1] *Manuscript received March 29, 1946.*

Contribution from the Institute of Parasitology, Macdonald College, McGill University, and the Division of Animal Pathology, Science Service, Dominion Department of Agriculture, with financial assistance from the National Research Council of Canada.

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readily entered by the contents of the other parts of the intestinal tract and if drugs are to reach the site of *E. tenella* infection via the intestinal tract the therapeutic effect would be very uncertain. Thus it became apparent during the experiments in chemotherapy that sulphamerazine was reaching the caecal pouches via the blood stream. The question arose as to whether the structure or the concentration of the drug was responsible for inhibiting the parasite and in order to elucidate this point a series of observations on the fate of certain sulphonamides in the intestinal tract of the chicken have been made.

Experimental Methods

The birds used were male Barred Plymouth Rocks, approximately 2½ lb. in weight. In the experiments involving dosing per os each bird was given 75 mgm. of the appropriate drug in a hard gelatine capsule, which, in some cases, included a small amount of gentian violet as a tracer. In experiments involving intravenous medication a 5% aqueous solution of the sodium salt of the drug was injected via the wing veins.

In some experiments the caeca of the birds were ligated close to the ileocaecal sphincter; this was done, with catgut, under ether anaesthesia and through an incision made through the abdominal wall anteroventral to the cloaca and slightly to the right side. Care was taken to avoid the inclusion of mesenteric blood vessels in the ligature. The drug was administered following recovery from the anaesthetic.

All birds were killed by stunning and bleeding, usually four hours after the drug had been administered. The intestines were removed, the serous surfaces washed free of blood, and the contents expressed by gentle massage. Blood was collected when the birds were killed.

The small intestinal and caecal contents were dried at 110° C., weighed, and ground finely.

Representative samples were taken and extracted with hot *N*/10 sodium hydroxide. The mixture was centrifuged and the supernatant withdrawn. This extraction was repeated three times and the supernatants pooled. This procedure was found to remove completely all the sulphonamides present, whereas neither water nor 95% ethyl alcohol was satisfactory in this respect. Continuous extraction in a Soxhlet extractor, as recommended for tissues by Bratton and Marshall (1), was found to be unnecessary. The combined extracts were made up to a suitable volume, and aliquots were appropriately diluted for sulphonamide determination.

The method used was that of Bratton and Marshall (1), in which diazotization followed by coupling with *N*(1-naphthyl) ethylenediamine dihydrochloride in acid solution produces a red dye. The intensity of this colour was measured in a Klett-Summerson photoelectric colorimeter using green filter No. 54. Blank determinations were made simultaneously by omitting the sodium nitrite from the mixture of reagents.

Determinations on whole blood were carried out as follows: 1 ml. of whole blood was laked in 13 ml. of distilled water, and after three minutes 6 ml. of 15% trichloracetic acid was added with thorough shaking. The precipitate was left for five minutes to settle, after which it was filtered off through a Whatman No. 42 filter paper. The filtrate, representing a 1 in 20 dilution was further diluted if necessary, and the total and free sulphonamide was determined according to the method of Bratton and Marshall. The use of 20% *p*-toluenesulphonic acid instead of 15% trichloracetic acid, as recommended by Morris (10), was found to yield identical results.

The factors for the various sulphonamides used, as determined on pure standard solutions, were as follows:—

Sulphanilamide,	1.00.
Sulphamerazine,	1.39.
Sulphapyridine,	1.46.
Sulphathiazole,	1.47.

Experimental Observations

Some preliminary observations were first made on two chickens that had been dosed four hours before death with 75 mgm. of sulphamerazine. It was found that a quantity of the drug was present in the caecal pouches and although these tests were not considered quantitatively accurate it was apparent that the amount in the caeca approximated that in the small intestine. These tests were immediately repeated, but this time two birds in which the caeca were ligated were used. Again an appreciable amount of the drug was detected in the caeca, but technical difficulties made a quantitative estimation unreliable.

Following further work by one of us (G.H.B.) on the method of analysis, a series of birds was studied.

Over a period of time seven birds were dosed with 75 mgm. of sulphamerazine per os and were killed four hours after dosing. There was a great variability between the amounts of the drug detected in the intestinal tract; the caeca contained an average of 0.33 mgm. (σ = 0.37) while the small intestinal contents contained 1.03 mgm. (σ = 0.77). The complete data are shown in Table I.

Owing to the low values recorded in the above tests five undosed birds were killed and the small intestinal and caecal contents were analysed for blank values. It was thus found that an average of about 27% of the previous estimation of the caecal drug content could be accounted for by the blank values in that part of the intestinal contents (see Table II). However, it was obvious from the analyses of the intestinal contents of the birds dosed with sulphamerazine that some of the drug had entered the caeca via the blood stream, as in the last four of the birds a gentian violet tracer had been used and this had in no case reached the point of junction of the ileum and caeca.

TABLE I

ANALYSIS OF INTESTINAL CONTENTS AND BLOOD FOUR HOURS AFTER DOSING WITH 75 MGM. OF SULPHAMERAZINE PER OS

Bird No.	Drug in small intestine		Drug in caeca		Drug concentration in blood at death (mgm. %)[1]	
	Mgm.	Mgm./gm. contents	Mgm.	Mgm./gm. contents	Free	Total
2	0.48	0.51	1.17	1.04	Not taken	
7	2.10	1.00	0.04	0.18	2.56	2.86
9	2.20	2.00	0.06	0.27	4.50	5.10
10	0.38	0.40	0.18	0.29	3.80	3.90
11	0.105	0.32	0.08	0.13	—	—
18	0.85	0.19	0.33	0.46	3.70	3.70
19	1.10	0.12	0.42	0.35	2.70	2.73
Average	1.03	0.65	0.33	0.39	3.45	3.66
Standard deviation	0.77	0.61	0.37	0.28	0.73	0.85

[1] *Mgm. % = mgm. per 100 cc. of blood.*

TABLE II

ANALYSIS OF INTESTINAL CONTENTS OF UNDOSED BIRDS FOR BLANK VALUES

Bird No.	Small intestine blank values		Caeca—blank values	
	Mgm.	Mgm./gm. contents	Mgm.	Mgm./gm.
1 *B*'	0.030	0.010	0.036	0.150
2 *B*	0.016	0.010	0.029	0.033
3 *B*	0.090	0.050	0.040	0.087
4 *B*	0.140	0.026	0.180	0.140
5 *B*	0.210	0.029	0.040	0.120
Average	0.097	0.025	0.065	0.106
Standard deviation	0.070	0.015	0.058	0.042

Other sulphonamides were similarly tested. Sulphathiazole in 75 mgm. doses per os, given to similar birds, which were also sacrificed four hours later, likewise was detected in the caeca. On the basis of amount of drug per gram of intestinal contents the results were comparable to those obtained with sulphamerazine (Table III).

Intravenous medication with the sodium salt of sulphamerazine was conducted in order to compare results with those obtained from dosing per os. As shown in Table IV, in five birds the amounts of drug in the caeca were approximately the same as those detected after oral dosing. There was some drug in the contents of the small intestine but this was significantly less than that in the birds dosed per os.

TABLE III

ANALYSES OF INTESTINAL CONTENTS AND BLOOD AFTER COMPARABLE DOSES OF SULPHATHIAZOLE (75 MGM. PER OS—FOUR HOURS)

Bird No.	Drug in small intestine		Drug in caeca		Drug concentration in blood at death (mgm. %)	
	Mgm.	Mgm./gm. contents	Mgm.	Mgm./gm. contents	Free	Total
2 *C*	0.650	0.96	0.17	0.21	—	—
7 *C*	0.160	0.38	0.36	0.80	1.11	1.14
9 *C*	0.255	0.85	0.28	0.44	0.70	0.70
10 *C*	0.233	1.17	0.04	0.23	12.00	12.10
11 *C*	0.525	1.01	0.068	0.39	—	—
Average	0.365	0.874	0.184	0.414		
Standard deviation	0.189	0.242	0.126	0.212		

TABLE IV

ANALYSES OF INTESTINAL CONTENTS AND BLOOD FOUR HOURS AFTER INTRAVENOUS ADMINISTRATION OF 60 MGM. OF SODIUM SULPHAMERAZINE

Bird No.	Drug in small intestine		Drug in caeca		Drug concentration in blood at death (mgm. %)	
	Mgm.	Mgm./gm. contents	Mgm.	Mgm./gm. contents	Free	Total
14	0.135	0.05	0.46	0.26	0.8	0.7
15	0.340	0.10	0.26	0.48	1.75	1.75
16	0.253	0.09	0.51	0.45	1.91	1.87
17	0.280	0.13	0.18	0.40	4.00	4.35
20	0.250	0.25	0.103	0.14	—	—
Average	0.252	0.124	0.303	0.346		
Standard deviation	0.069	0.068	0.160	0.300		

Comparable tests were then conducted using sulphanilamide, sulphapyridine, sodium sulphathiazole, and sodium sulphapyridine, the last two being administered intravenously. These limited tests revealed amounts of drug in the caeca and intestine similar to those recorded for sulphamerazine (Table V).

The caeca of three birds were ligated and the birds were later dosed with 75 mgm. of sulphamerazine per os and killed four hours later. The analyses showed that the contents of the small intestine contained an average of 0.35 mgm. (0.29 mgm./gm. contents) of drug and those of the caeca 0.1 mgm. (0.42 mgm./gm. contents). It was evident in these tests that the normal movement of ingesta had been considerably retarded by the manipulation of

TABLE V

CHECK TESTS OF VARIOUS SULPHONAMIDES : ANALYSES OF INTESTINAL CONTENTS

Bird No.	Drug and dosage	Drug in small intestine		Drug in caeca		Drug concentration in blood at death (mgm. % at four hours)	
		Mgm.	Mgm./gm. contents	Mgm.	Mgm./gm. contents	Free	Total
18 *A*	SA., 75 mgm. per os	0.440	0.114	0.097	0.187	1.11	4.11
18 *B*	SP., 75 mgm. per os	0 396	0.152	0 376	0.775	0 42	0.59
17 *B*	NaST., 60 mgm. intrav.	0.470	0.330	0 860	0.950	0.49	0.45
22	NaSP., 60 mgm. intrav.	0.367	0.106	0.46	0.363	0.41	0.51

NOTE: *SA.* = *sulphanilamide.*
SP. = *sulphapyridine.*
NaST. = *sodium sulphathiazole.*
NaSP. = *sodium sulphapyridine.*

the intestines, as the visible tracer was still largely in the crop and gizzard. However, the amount per gram of caecal contents, although small, was 10 times more than the expected blank value.

In most of our tests we set the dose at 75 mgm. and the time between dosing and killing the birds at four hours. In a total of 13 birds, however, using sulphamerazine and sulphathiazole, the doses and times were varied for purposes of comparison. It was found (Table VI) that the amounts detected in the two organs were still very variable and did not follow expected trends.

Observations on Sulphonamide Concentrations in the Blood

The routine analyses of blood at the time of slaughter of many of the birds revealed great variability in drug concentrations (Tables I, III, IV, V, VI). There was no apparent correlation between the amount of drug in the blood and that in the intestines in these tests. One point of interest is that neither sulphamerazine nor sulphathiazole was conjugated to any extent, but a large proportion of the sulphanilamide and a small proportion of the sulphapyridine were conjugated. These findings of low or absent conjugation of sulphamerazine in chickens is in agreement with the more detailed work of Welch, Mattis, Latven, Benson, and Shiels (14).

Discussion

The method of collecting the intestinal contents precluded the possibility that our material for analysis contained mucosa or blood in amounts that could be detected in our results. Thus it seems certain that the amounts found in the caecal contents represent excreted drug. It is also apparent

TABLE VI

VARIATIONS IN DOSAGE AND TIMES OF KILLING BIRDS; DRUG LEVELS IN INTESTINAL CONTENTS AND BLOOD

Bird No.	Drug and dosage	Time, dose to slaughter (hr.)	Drug in small intestine		Drug in caeca		Drug concentration in blood at death (mgm. %)	
			Mgm.	Mgm./gm. contents	Mgm.	Mgm./gm. contents	Free	Total
19	SM. 75 mgm. per os	4	1.10	0.12	0.42	0.35	2.70	2.73
19 *A*	SM. 150 " " "	4	0 96	0.29	0.54	0 58	5.20	5.55
19 *B*	SM. 300 " " "	4	3.82	1.91	0.64	0.90	11.30	11.80
5	SM. 75 " " "	1	1.34	2.20	0.28	0.28	3.08	2.86
4	SM. 75 " " "	2	4.57	1.87	0.21	0.58	—	—
6	SM. 75 " " "	2	1.63	1.25	0.27	0.41	9.1	8.9
3	SM. 75 " " "	3	1.16	0.45	1.01	0.63	—	—
8	SM. 75 " " "	3	2.15	2.13	0.05	0.42	6.20	6.70
5 *A*	ST. 75 " " "	1	1.38	0.97	0.25	0.25	2.02	1.93
4 *A*	ST. 75 " " "	2	0.53	0.25	0.41	0.36	—	—
6 *A*	ST. 75 " " "	2	0.75	0 46	0.04	0.26	1.58	1.64
3 *A*	ST. 75 " " "	3	0.59	0.39	0.57	0.30	—	—
8 *A*	ST. 75 " " "	3	0 38	1.28	0.04	0.17	1.47	1.54

NOTE: *SM.* = *sulphamerazine.*
ST. = *sulphathiazole.*

from the results obtained from intravenous medication that sulphonamides are excreted in the small intestine, as well as in the caeca, but to a somewhat lesser extent.

It is possible that continuous dosing in feed would have resulted in a higher concentration in the caeca over a period of time, but, as the results of our tests had to rely on the fact that the drug could not have entered the caeca from the lumen of the small intestine, the administration of a single dose was the only practical method. However, as this work was designed primarily to find out whether the coccidiostatic action of the sulphonamides was due to drug concentration or drug structure, the experiments showing that sulphonamides other than the coccidiostatic sulphamerazine also appear in the caecal contents appear to have achieved that end. If we had demonstrated that only the sulphonamides that possess the pyrimidine structure possessed the property of being excreted via the caeca, this finding would have suggested that drug concentration rather than structure was responsible for inhibiting some stage of the protozoan parasite, *Eimeria tenella*, in the caeca. It is now apparent that the inhibitory effect on the parasite is probably due to drug structure rather than to any peculiar property of a drug that allows concentration at the site of infection. A full understanding of the coccidiostatic effect of the drugs might rely upon the finding of a specific metabolite used by the merozoites of *E. tenella*, with which the pyrimidine-sulphonamides possibly compete.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 25, SEC. D. AUGUST, 1947 NUMBER 4

PHYLLODISTOMUM LACHANCEI SP. NOV., A TREMATODE FROM THE URETERS OF *SALVELINUS FONTINALIS* (MITCHILL), WITH A NOTE ON ITS PATHOGENICITY[1]

BY L. P. E. CHOQUETTE[2]

Abstract

A new species of trematode (Gorgoderidae), *Phyllodistomum lachancei*, collected from speckled trout, *Salvelinus fontinalis* (Mitchill), in Montmorency County, Que., is described. The presence of this parasite in the ureters of the host produces at most only slight pathological changes.

In the course of the examination of speckled trout, *Salvelinus fontinalis* (Mitchill), collected in August, 1945, in the northern part of Quebec, specimens of a trematode were found in the ureters by Mr. F. Lachance of this Institute. The worms were present in small numbers only, not more than eight being recorded in any one fish. The flat edges of the worm were unrolled so that the parasite practically occluded the tube and, to the naked eye, presented the appearance of a small abscess. They could be removed from their habitat by gently pressing along the length of the tube in the direction distal from the kidneys. The flukes when expressed into water were very active, the anterior end being extended and bent as though it were a proboscis. This appearance is not evident, however, in the fixed specimens.

This parasite was found to belong to the genus *Phyllodistomum* Braun, 1899, and further studies showed it to be a hitherto undescribed species. The following description is based upon the study of mounted and unmounted specimens fixed in 70% alcohol, stained in alum carmine, and mounted in Canada balsam. Section of the kidney showing the worms *in situ* was also made.

Description

The body (Fig. 1) consists of two portions: an anterior one, elongated and distinctly set off from a wider, spatulated posterior one. The margins are smooth and the posterior end is notched. The average length of mounted specimens is 2.94 mm. while the average width taken at the level of space between the anterior margin of posterior testis and posterior margin of anterior testis is 1.31 mm. The terminal oral sucker is smaller than the acetabulum

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[1] *Manuscript received April 3, 1947.*

Contribution from the Institute of Parasitology, Macdonald College, McGill University, with financial assistance from the National Research Council of Canada.

[2] *Research Assistant.*

[The June issue of Section D (Can. J. Research, D, 25 (3): 97-129. 1947) was issued August 14,1947.]

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situated at the junction of the anterior and posterior portions of the body. The ratio of the diameter of the oral sucker to that of the acetabulum is as 1 : 1.24.

The length of the oesophagus is less than the length of the oral sucker. The intestinal caeca extend to the posterior end of the body. The vitellaria are oval and smooth, although occasionally, in immature specimens, indented vitellaria are seen; they are posterolateral and very close to the acetabulum, sometimes overlapping it. The vitelline ducts join medially. The shell gland lies between the vitelline glands in front of the common vitelline duct.

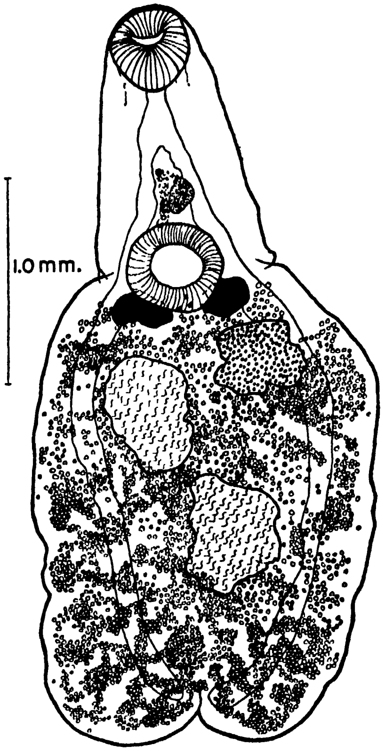

FIG. 1. *Phyllodistomum lachancei, adult, ventral view.*

The ovary is irregularly shaped, strongly lobate or faintly indented on either side, amphitypic; it is smaller than either testis and lies very close to the vitellarium of the same side. The uterus is voluminous and contains a great number of eggs; the uterine coils, intra- and extra-caeca run backwards to the posterior margin of the body and forward to the anterior margin of the ovary. The testes are obliquely placed, fairly close to each other, elongated or roundish, irregularly lobate or indented. The distance between the testes is less than the lesser dimension of either testis. The testes lie in the middle of the posterior part of the body, the anterior one being behind and oblique to the ovary. The seminal vesicle is quite large and dilated posteriorly.

The genital pore is situated ventrally in the space between the intestinal bifurcation and the anterior margin of the acetabulum. The excretory pore is terminal and lies at the posterior notch.

Measurements are given in millimetres, with the average in parentheses, of 25 carmine-stained specimens, mounted in Canada balsam. Body: length, 2.01–3.68 (2.92); width, at level of space between posterior margin of anterior testis and anterior margin of posterior testis, 0.96–1.49 (1.31). Oral sucker: diameter, 0.22–0.36 (0.278); acetabulum: diameter, 0.24–0.42 (0.346). Oesophagus: length, 0.105–0.181 (0.144). Right vitellarium: length and width, 0.07–0.22 by 0.10–0.27 (0.15 by 0.19); left vitellarium: length and width, 0.07–0.18 by 0.12–0.25 (0.13 by 0.19). Ovary: length and width, 0.16–0.39 by 0.15–0.36 (0.27 by 0.23). Testes—anterior: length and width, 0.31–0.74 by 0.19–0.54 (0.50 by 0.41); posterior: length and width, 0.36–0.75 by 0.30–0.57 (0.53 by 0.42); distance between anterior and posterior testis, 0.06–0.45 (0.18). Older intrauterine eggs obtained by dissection of the worm measure 0.03–0.05 by 0.02–0.03 (0.04 by 0.02).

Comparison of this species with descriptions of all the other known species of *Phyllodistomum* shows that it differs from all of them by the presence or absence of one or more morphological characters. By having a body definitely divided into two portions it differs from: *P. acceptum* (6), *P. conostomum* (6), *P. elongatum* (6), *P. lesteri* (15), *P. linguale* (6), *P. marinum* (6), *P. nocomis* (3), *P. parasiluri* (16), *P. sinense* (14). An oral sucker smaller than the acetabulum distinguishes it from *P. brevicecum* (13), *P. caudatum* (12), *P. etheostomae* (4), *P. morgurndae* (16), *P. pearsei* (5), and *P. unicum* (6). The size, shape, and position of the gonads distinguish it from *P. almorii* (10), *P. americanum* (6), *P. angulatum* (6), *P. carolini* (5), *P. entercolpium* (6), *P. fausti* (6), *P. hunteri* (1), *P. kajika* (2), *P. lacustri* (6), *P. lohrenzi* (7), *P. lysteri* (9), *P. macrobrachiola* (16), *P. macrocotyle* (6), *P. megalorchis* (6), *P. nocomis* (3), *P. notropidus* (3), *P. patellare* (6), *P. pseudofolium* (6), *P. semotili* (3), *P. shandrai* (2), *P. singulare* (8), *P. solidum* (11), *P. spatula* (6), *P. spatulaeforme* (6), *P. staffordi* (6), and *P. undulans* (13). It resembles most closely *P. folium* (6) and *P. simile* (6). It differs from the former by its length and width, size of oral sucker and acetabulum, shape and position of the testes, the extent of the uterine coils and size of eggs; it differs from the latter by its size, its larger vitellarium, the shape and position of the testes and size of eggs. Its smooth margins distinguish it from *P. superbum* (6) in which the margins are heavily crinkled. Therefore, it is considered that these differences are sufficient to separate the present specimens from the species described previously in this genus and accordingly the name *Phyllodistomum lachancei* is proposed for it.

Host: *Salvelinus fontinalis* (Mitchill).

Habitat: Ureters.

Locality: Montmorency County, Province of Quebec, Canada.

Type and co-types: British Museum (Natural History), Cromwell Road, London, S.W. 7, England.

Pathogenicity

The presence of this parasite in the ureters produces at most only slight pathological changes. There is a dilation of the ureters with some flattening of the lining columnar epithelial cells (Figs. 2 to 5). From sections studied there is no evidence of damage to the parenchymal tissue.

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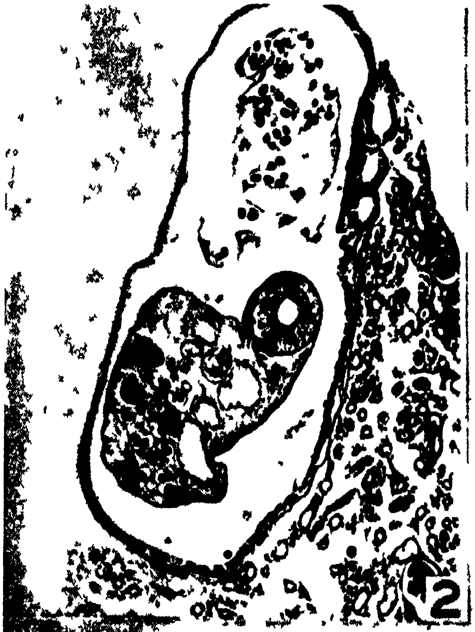
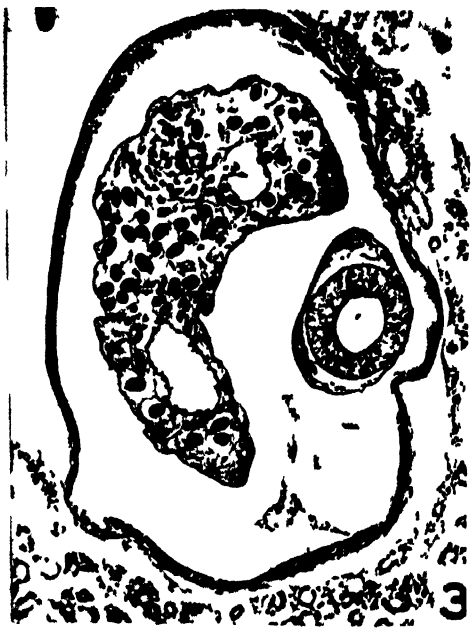
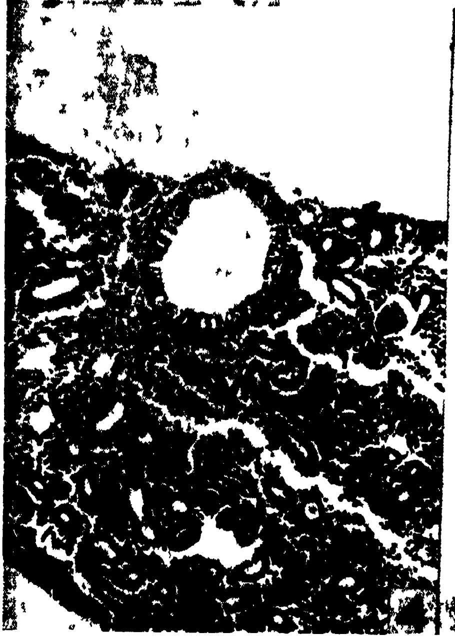
PLATE I







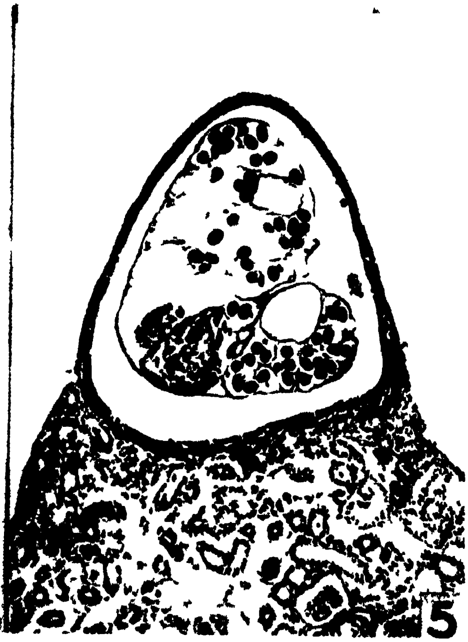


FIG. 2 *Photomicrograph of section of Phyllodistomum lachancei in situ* ×36

FIG. 3 *Photomicrograph of section of Phyllodistomum lachancei in situ* ×60

FIG. 4 *Photomicrograph of section of normal ureter of Salvelinus fontinalis* ×60

FIG. 5 *Photomicrograph of section of Phyllodistomum lachancei in situ* ×60

Canadian Journal of Research

Issued by The National Research Council of Canada

VOL. 25, SEC. D. OCTOBER, 1947 NUMBER 5

THE PROVENTRICULAR REGION OF *MACHILIS VARIABILIS* SAY (THYSANURA : MACHILIDAE)[1]

By W. W. Judd[2]

Abstract

The structure of the proventricular region of the bristle-tail, *Machilis variabilis* Say, is described. Its structure is found to be intermediate in complexity between that of the simple, tubular fore-gut of the Collembola and that of the muscular proventriculus of the silver-fish, *Lepisma*.

Materials

Ten mature specimens (length of body, 1.0—1.2 cm.) of the bristle-tail, *Machilis variabilis* Say, were collected from beneath rocks in woods in the vicinity of Ottawa, Ont. on Sept. 2, 1939. They were identified with the aid of the keys and descriptions of MacGillivray (5) and Lubbock (4).

Each insect was opened carefully, with eye scissors, along the mid-dorsal line, and was then placed in Bouin's fixative for about six hours. After fixing and clearing, transverse sections (10μ) were made of the whole insect. Sections were stained with haemotoxylin and eosin. Shrinkage of tissues was slight; the diameters of the prothorax (1.5 mm.) and mesothorax (2.0 mm.) after sectioning were the same as in untreated specimens.

The following description of the proventricular region, including the measurements of the various tissues, is based on serial sections of two specimens of the bristle-tail.

Literature

The structure of the fore-gut of various species of Thysanura and Collembola has been studied by several authors. MacNamara (6) and Lubbock (4) point out that the majority of Collembola live on decaying vegetable matter, moulds, and algae, and the latter author shows that the digestive canal is essentially a straight tube. Descriptions of the fore-gut of various species of Collembola (1, 7, 9) show that it consists of a narrow oesophagus leading into the mid-gut by a small oesophageal valve. The proventricular region shows no features to distinguish it from the rest of the fore-gut.

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[1] *Manuscript received May 16, 1947.*

Contribution from the Department of Applied Biology, University of Western Ontario, London, Ont. Part of a thesis submitted for the degree of Master of Arts of the University of Western Ontario.

[2] *At the time, Demonstrator in Biology, University of Western Ontario; now Lecturer in Zoology, McMaster University, Hamilton, Ont.*

[The August issue of Section D (Can. J. Research, D, 25 : 131-134. 1947.) was issued October 6, 1947.]

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Newcomer (8) studied the digestive system of the silver-fish, *Lepisma*, and speaks of it as "including a remarkable muscular crop, the posterior end of which protrudes into the mid-intestine". Gray (2) describes this crop as "a spherical gizzard provided with six teeth". Lubbock (4) also points out that the "crop" has six horny teeth. It is thus evident that the gizzard (proventriculus) is a well developed structure in *Lepisma*.

Description

In *Machilis variabilis* Say the oesophagus occupies the prothorax and mesothorax (Fig. 1 – Oes.). It is surrounded by four lobes projecting forward from the mid-gut (L). These lobes are similar in size and position to the four gastric coeca in the alimentary canal of *Petrobius* described by Imms (3). In the prothorax the oesophagus is about 130μ in diameter and is surrounded by a single sheath of circular muscle 3-4μ thick (Fig. 2 – CM). The epidermal layer is composed of a single layer of cells roughly cuboidal in shape and 5-10μ thick (EP). The sclerotized intima (I) is composed of two layers, an outer one ranging from 1 to 10μ in thickness, and an inner one of a constant thickness of 1μ. It is thrown up into longitudinal ridges of irregular shape. In the posterior part of the prothorax the diameter of the oesophagus is about 140μ and the sheath of circular muscle surrounding it is about 1μ thick (Fig. 3 – CM). The ridges of the intima in the lateral walls of the oesophagus are flattened, while dorsally and ventrally the intima projects into the lumen, forming dorsal and ventral ridges (Fig. 3 – DR, VR), which almost fill the lumen.

In the fore part of the mesothorax the posterior end of the oesophagus forms the oesophageal valve (Fig. 4 – Oes. V.). It is surrounded by two lobes of the mid-gut (L). The vertical diameter of the valve is about 180μ and the transverse diameter about 90μ. In this region the dorsal and ventral ridges of the oesophageal valve touch one another and are broadened laterally to touch the lateral walls. Consequently the lumen is almost obliterated (Fig. 5). A transverse section of the oesophageal valve (Fig. 5) shows, on the outside, the irregular layer of intima (Iii). Inside this layer are two layers of epidermis (EP) separated by narrow bands of circular muscle (CM). The outer layer is continuous with the much larger cells of the mid-gut (MG). The inner layer supports the inner sclerotized intima of the valve (Ii).

FIG. 1. *Transverse section of the prothorax.*

FIG. 2. *Transverse section of the oesophagus in the prothorax.*

FIG. 3. *Transverse section of the oesophagus in the posterior part of the prothorax.*

FIG. 4. *Transverse section of the mesothorax.*

FIG. 5. *Transverse section of the oesophageal valve.*

CM—Circular muscle; DR—Dorsal ridge of oesophagus; EP—Epidermis; I—Intima of oesophagus; Ii—External intima of oesophageal valve; Iii—Internal intima of oesophageal valve; L—Lobe of mid-gut; MG—Cells of mid-gut; Oes.—Oesophagus; Oes. V.—Oesophageal valve; VR—Ventral ridge of oesophagus.

Fig.1

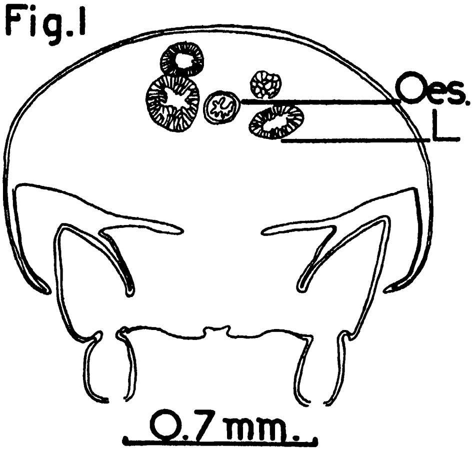


Fig.2

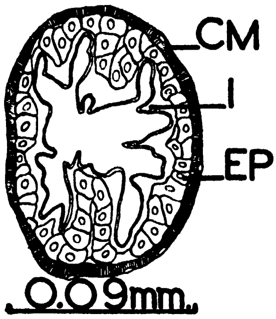


Fig.3

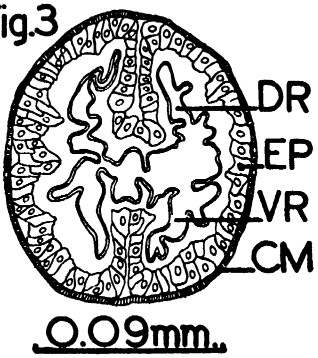


Fig.5

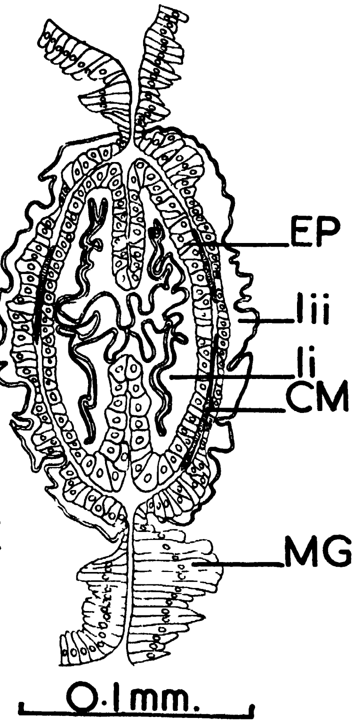
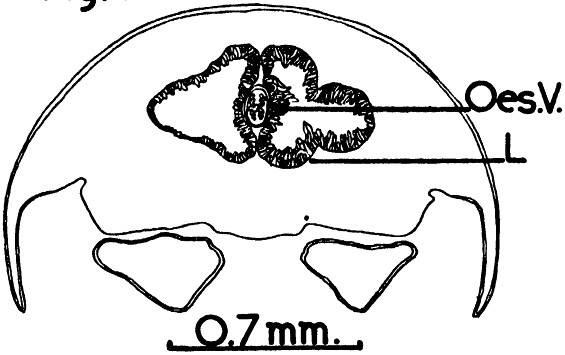


Fig.4



The foregoing description indicates that the proventricular region of *Machilis variabilis* Say is intermediate in complexity of structure between that of the Collembola and that of *Lepisma*. In the former the proventricular region is not to be distinguished from the tubular oesophagus. In the latter the proventriculus is a well defined structure bearing six horny teeth. In *M. variabilis* the proventricular region shows no abrupt separation from the rest of the fore-gut, but bears internally strong dorsal and ventral ridges that almost completely obliterate the lumen.

Acknowledgment

The writer wishes to express his gratitude to Prof. J. D. Detwiler for his guidance in this work.

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THE TIMBER WOLF IN THE ROCKY MOUNTAIN NATIONAL PARKS OF CANADA[1]

BY IAN MCTAGGART COWAN[2]

Abstract

The wolf (*Canis lupus occidentalis*) population of the Rocky Mountain National Parks of Canada, an area of 7000 square miles of mountainous terrain, was studied from 1943 to 1946. During that time it approximated a density of one wolf to between 87 and 111 square miles of potential range, which was reduced to about 10 square miles per wolf during late winter by contraction of available range. The range is heavily populated with the wild ungulates, *Ovis canadensis*, *Oreamnos americanus*, *Alces americana*, *Rangifer arcticus*, and *Odocoileus hemionus*. Many of the winter ranges are grossly overstocked. The annual diet of wolves in the area has consisted of 80% big game, with elk alone contributing 47%. Mule deer contribute another 15%. Although bighorn sheep are in abundance and available they are little hunted by wolves. Eighteen per cent of the annual diet consists of rodents, of which snowshoe hare and beaver are the two most important. Wolves are unevenly distributed and many game herds have been living without wolf pressure. Comparison of the vital statistics of game herds living with and without wolf predation reveals no discernible difference in the survival of young or in the sex ratios within the two groups. It is concluded that under existing circumstances the wolves are not detrimental to the park game herds, that their influence is definitely secondary, in the survival of game, to the welfare factors, of which the absence of sufficient suitable winter forage is the most important.

In the spring of 1943, when I began a series of wildlife studies in the Rocky Mountain National Parks of Alberta and British Columbia, I was given to understand that the apparent increase in the numbers of predatory mammals in these parks was arousing concern among certain groups interested in the ungulate mammal populations of the parks.

Hand in hand then with the gathering of data upon the ungulate mammals of the parks went the painstaking and often tantalizing task of gathering data relative to carnivores. The information upon wolves and their activities in the parks accumulated during the studies is presented herewith.

The study of wolves in the heavily timbered mountainous terrain that makes up so large a part of the area of the Rocky Mountain Parks of Canada presents many difficulties. One seldom sees the animal that is the main subject of his inquiries and must perforce be content with data gleaned from sources other than direct observation of the principals. Tracks there are aplenty, kills can be found and scats collected, and it is upon these and similar sources of data that one must depend in the main.

The investigation of wolves therefore fell into several different phases.

1. To determine what the wolves were eating, scats were collected and analysed.

Manuscript received April 19, 1947.

Contribution from The Department of Zoology, University of British Columbia, Vancouver, B.C., and National Parks Bureau, Department of Mines and Resources, Ottawa, Canada.

[2] *Professor of Zoology, University of British Columbia.*

2. In an attempt to qualify the information obtainable from scat analysis special effort was made to determine the circumstances surrounding the killing of certain species and individuals among the ungulate and rodent populations of the park. Kills were examined wherever possible and data secured on the age class and probable physical condition of the victim.

3. Tracks were followed and the behaviour of the wolves studied as it was betrayed by their footprints.

4. In an attempt to arrive at a reasonably accurate estimate of the year by year population of wolves in the parks and their seasonal movements the good offices of the various park wardens were sought. Many of these men went out of their way to obtain counts of individuals in different parks and to chart the movements of these groups in their respective districts.

5. Certain of the wardens either by reason of peculiar local conditions or more than ordinary personal enthusiasm were able to make first hand observations that contributed importantly to our information on the wolf and its ways. I have drawn extensively upon the factual information gleaned from these men and have acknowledged all such material wherever it is used.

These have been the sources of the direct information on wolf populations and behaviour, subjects vital to the proper understanding of the position of the wolf in the biological society of which it is probably the most important influent. Equally important, in the present instance, is the appraisal of the over-all influences of wolves upon the prey species. The local circumstances in the Rocky Mountain parks offered one useful possibility of examining this latter circumstance. Coyotes, mountain lions, and grizzly bears were found to be fairly evenly distributed over most of the areas under study. The larger ungulates were with few exceptions widely distributed. Wolves on the other hand are absent from some large areas—abundant in others. It was possible then to study populations of many of the ungulates living under approximately equivalent circumstances except for the presence or absence of wolves. Selected areas presenting these contrasting circumstances were given special study and the vital statistics of game herds living with and without wolf pressure assembled for comparison and study.

The Study Area

The Rocky Mountain Parks of British Columbia and Alberta consist of Jasper Park, with an area of 4200 sq. miles; Banff Park, 2400 sq. miles; Kootenay Park, 527 sq. miles; Yoho Park, 507 sq. miles; and Waterton Lakes Park with an area of 286 sq. miles. The first four of these occupy contiguous territory, the two first on the Alberta side and the two latter on the British Columbia side of the interprovincial boundary. Together they constitute the largest single reserved area on the North American continent. They extend the full width of the main range of the Rocky Mountains from the region of the 51st parallel to 53° 30′ north latitude. Waterton Lakes Park, the smallest of the five, is situated in the southwesternmost corner of Alberta, with its southern boundary on the 49th parallel of latitude.

The wolf population of Waterton Lakes Park is so small that it is unimportant in the present study.

The entire study area lies in the floral association leading to a spruce forest climax through various seral types, which, as forests, begin as jack pine or aspen stands at lower elevations, balsam forests at and near timber line. Elevations above 7000 ft. are for the most part above tree line. Here the spruce and balsam forests give way abruptly to extensive alplands upon which the lower areas support a heavy growth of a half a dozen or more species of willows (*Salix*). These become progressively shorter in stature with altitude and give way to the heaths (*Phyllodoce* and *Cassiope*), the crowberry (*Empetrum nigrum*), and many flowering plants, short grasses, and sedges at still higher levels.

Along certain of the lower valleys, such as the Athabaska and Brazeau in Jasper, the Red Deer, Panther, and Bow in Banff, there are patches of grassland usually with greatly varied relief. Open, flat land is virtually nonexistent. Rockslides and lakes are abundant at all elevations.

The lowest elevations in Jasper Park are about 3300 ft., those in Banff almost 1000 ft. higher. The snow lies late in the spring and for this reason, wolf denning takes place in the timbered areas of the park and by far the largest part of the wolf's life is spent in country in which long range visibility is impossible.

Two races of wolves contributed to the population of the area under study during the early period of its invasion by white men. The areas now included in Jasper Park probably supported a population of the Mackenzie Valley wolf (*Canis lupus occidentalis* Richardson), while the Waterton Lakes area was inhabited by the Northern Rocky Mountain wolf (*Canis lupus irremotus* Goldman).

Specimens of *occidentalis* are listed by Young and Goldman (12) from Edmonton, and from 50 miles SW. of Smith Landing, Alta., and it is probable that the contiguous Rocky Mountain region was inhabited by the same race.

Southern Alberta, north to Calgary, was within the range of *irremotus* and Young and Goldman (12, p. 448) list specimens from Calgary, Gleichen, Lethbridge, and 25 miles southeast of Lethbridge. It can safely be assumed that this was the race entering the Waterton Lakes area.

As will be detailed later, wolves were exterminated in the latter region about 1922 and are but now reappearing. The Northern Rocky Mountain wolf (*irremotus*), as compared with the Mackenzie wolf (*occidentalis*), was a smaller animal, with significantly smaller skull and lighter teeth. It was also a 'grey' wolf, and gave rise to very few black individuals.

Occidentalis, on the other hand, is a larger wolf producing many blackish or black individuals.

In the winter of 1943-44 one black wolf and in 1944-45 two wolves were known to be ranging in the area adjacent to and within the eastern edge of Waterton Lakes Park. One of these, a black male, was shot. The skull of this specimen has been available for study and proves to be just within the size range of *irremotus* in all but 2 of 15 measured features but in the sum of all characters to be referable to the race *occidentalis*. It would seem then, that the northern race has invaded the territory previously occupied by *irremotus* and that the current recrudescence in the wolf population of the Rocky Mountains does not reflect a reappearance of the latter race. It is unlikely that the race was completely extirpated, but it may well have been so reduced in numbers in the foothills and mountains of southwestern Alberta through persistent hunting that *occidentalis*, developing an augmented population pressure in this or the last cyclic peak, has inundated the remaining population of *irremotus* and thus extirpated the race.

Corroborative evidence of this is indicated by the characteristics of the only specimen taken in recent years in that part of the former range of *irremotus* south of the 49th parallel. This animal, taken in Gallatin County, Montana, in 1941, is noted by Young and Goldman to be as large as individuals of *occidentalis* except for lighter dentition. The published measurements of the skull of this specimen reveal that even the dentition comes within the normal range of variation in *occidentalis*. There are therefore no grounds for referring this specimen to any other race. It is suggested therefore that this individual was merely the southernmost wanderer from the current invasion from the north and was indeed a specimen belonging to the race *occidentalis*.

Five specimens from the Yellowhead Pass area of British Columbia and Alberta in the Museum of Zoology, University of British Columbia, are *occidentalis*.

Physical Characteristics

The general form of the wolf is too well known to require description here; however, size data and information concerning colour and the proportions of various colour phases in the general population are scarce in the literature.

Several wolves were destroyed in Jasper Park during the years between 1942 and 1945 but for the most part these were disposed of before I was able to make any examinations.

Two young adult males taken at Decoigne, Alta., in 1944 were measured and weighed. External measurements of these were total length: 1880 mm., 1885 mm.; tail: 470 mm., 460 mm.; hind foot: 290 mm., and 280 mm. The larger of the two weighed 120 lb., the smaller 110 lb. A female shot in the same area on Apr. 13, 1944, was estimated to weigh approximately 80 lb.

A two year old female shot at Miette in November 1945 measured total length, 63 in.; tail, 18¼ in.; hind foot, 10¾ in.; ear, 4¾ in. An adult female shot at Decoigne on June 12, 1946, measured total length, 63 in.; tail, 17 in.; hind foot, 11 in.; ear, 4¼ in.; weight, 83 lb.; another adult female taken at Devona in December 1945 measured total length, 63 in.; tail, 18¼ in.; hind foot, 10¾ in.; ear, 4¾ in.

An adult male wolf shot in the same place on May 10, 1944, measured total length, 1825 mm.; tail, 475 mm.; hind foot, 275 mm.; and weight, 140 lb. with its paunch full.

A large wolf killed by Warden Frank Wells on Buffalo Prairie and weighed by him in the presence of several witnesses scaled 172 lb. This animal was distended with the load of elk meat in its stomach.

In September 1944 Warden White of Decoigne took a male pup weighing 70 lb., including a full stomach of elk meat. A female pup dug out of its den by Warden Chas. Page and examined by me on July 10, 1946, weighed 19 lb.

Colour

The colour of the wolves of the Rocky Mountain Parks is most variable, palest animals are almost white, darkest animals are almost black. The largest colour group is dark grey, the occasional individual quite brownish.

The grey and black colour phases do not seem to be mutually exclusive colours inherited en bloc. Some individuals are almost exactly intermediate between the two extremes.

A tabulation of the colours—either as grey or as black—of all wolves reported to me during the years 1944-46 reveals that, of 80 wolves seen by the various wardens of Jasper and Banff and by members of my party during this period, 44 or 55% were black and 36 or 45% grey.

This appears to be a higher proportion of blacks than hitherto reported in any North American subspecies of *Canis lupus* with the possible exception of *C. l. ligoni* from the islands of southeastern Alaska. It approximates the condition reported by Murie (9) in Mount McKinley Park, Alaska.

History

It is probable that wolves have always been present, in recent time at least, in small numbers, in certain parts of the study area. The earliest wolf record for the region concerns the great dire-wolf of Pleistocene time. In July, 1945, I picked up a fossilized tooth of this wolf among some gravel weathered from the higher peaks and lying near the middle of the Castleguard Icefield.

In more recent time the earliest explorers make casual mention of the presence of wolves in the Athabaska Valley northeast of Jasper.

At the time that serious biological studies in and adjacent to Jasper Park were begun, wolves were scarce. For example Loring, in the early autumn of 1896, reported (10) that he occasionally saw tracks of timber wolves in the country between Jasper House and the Smoky River. He heard one at Grand Cache River on Sept. 5.

Hollister (6) states that at the time of his visit to the Jasper region wolves were very scarce and reports Lewis Swift, long a resident at Henry House, as having killed but two in 19 years.

It is pertinent to note that the period in question was also one of great game scarcity. Elk were gone from the entire area now occupied by the Rocky Mountain Parks; moose, sheep, and deer were scarce; and only caribou and goat were present in fair numbers. These species usually winter at too great an altitude in this region to provide suitable winter food for wolves.

Authentic information on the changing status of wolves in this region in the period between 1912 and the present is difficult to obtain. Jack Hargreaves has supplied me with some figures obtained by himself and his brothers during many years in the Rocky Mountains adjacent to Jasper. He states that in 1914 two wolves appeared on the upper reaches of the Whirlpool River and ranged over the summit into the Fortress Lake area. In two years' time this band had become seven or eight individuals. They were subsequently poisoned.

In 1917 many wolves appeared along the Moose River and in this and the next year or two the population seemed to be near a peak. In 1918 Roy Hargreaves and a companion saw upwards of 40 wolves, most of them at one time, along the Wapiti River, a tributary of the Peace River, 150 miles or so map distance north of Jasper.

There is a gap in our data from 1918 to 1940. During this period, apparently no records were kept of wolf numbers or distribution in Jasper Park. Wolves were absent from Kootenay and Yoho Parks except for occasional travelling individuals. Evidence discovered in 1946 reveals that a pair of wolves had denned for at least eight years in the Panther River valley about six miles below the warden cabin.

In Jasper Park, at the present time, wolves are present during all seasons of the year along the Miette River from Yellowhead Pass eastward along the Athabaska from the junction of the Sunwapta to the confluence of the Moosehorn and Athabaska near the northern edge of the eastern boundary. From Honeymoon Lake to Snaring the wolves range on both sides of the river but below Snaring they are almost confined to the country west and north of the Athabaska. Just one wolf has been seen from time to time on the south side of this river on the area adjacent to its confluence with Rocky River.

Wolves occur up Whirlpool River during all seasons as they do also along the Brazeau and the lower reaches of the Snaring River and the Snake Indian River below Willow Creek.

Summer range, from which the wolves migrate during periods of deep loose snow in the winter months, includes the upper Snake Indian, Topaz Creek, Moose River, and the valley of the Smoky River.

There are extensive areas yet without a wolf population. For example there are no wolves along the valley of Rocky River, along the Maligne Valley above the canyon, along the Cairn or upper Southesk Rivers, in the Tonquin Valley, and at the head of the Athabaska above the Sunwapta.

Game is plentiful in several of these areas and it is difficult to explain the peculiarity of wolf distribution in these parts.

In Banff Park there is a small resident population along the Saskatchewan River and along the lower parts of the Howse and Mistaya Rivers. The first wolves to enter this region, two in number, did so in the winter of 1942-43.

In 1943 there was a small resident population in the vicinity of Ya Ha Tinda Ranch and along the Red Deer River from the ranch to Scotch Camp opposite the mouth of Divide Creek. Wolves made periodic incursions into other parts of the eastern edge of the park. In 1943 they ranged up the Panther as far as Windy Cabin at the mouth of Whigmore Creek but no sign of their presence on the Panther was noted in 1945. As already stated, in July, 1946, while investigating a wolf den near the Sulphur Springs in the Panther River Valley, we found other old and new dens from which it was obvious that there has been a pair of wolves denning in this valley for at least eight years, possibly much longer. In the winter of 1944-45 three individuals came up the Ghost River to Minnewanka Lake but returned without making a kill. In 1943, 1944, and 1945 single animals were seen at Bow Summit, Goat Creek, and in the Bow Valley 18 miles west of Banff. During the winter of 1945-46 a group of five or six wolves hunted the Clearwater Valley; in the spring they denned five miles below the Park boundary.

Wolves have apparently been rare on the western slope of the Rockies at least through the period for which there is any record available. As outlined by Munro and Cowan (7), there are but two records of the presence of wolves in Kootenay Park. In 1906 two wolves came up river from near Canal Flats and crossed through the park. Again, in 1936 or thereabouts, Mr. V. H. Lord heard wolves howling and saw one of them near Vermilion Crossing.

The only recent record for Yoho Park is that of a single individual reported to me by Park Warden Nicholson. This animal came up Beaverfoot River in the winter of 1942-43 and it—or another—was killed two or three days later farther down the Kicking Horse Valley, just outside the Park.

Information on the Waterton Lake area was obtained from F. H. Rigall of Twin Butte, Alta. Grey wolves used to be fairly common in the region. In 1921 increasing depredations upon domestic livestock led the stockmen to employ a noted wolfer, H. Rivière. As a result of this man's activities wolves were locally extirpated and did not reappear until 1943, when one black individual entered the area. In 1944 two black wolves were present but the male was shot at a beef carcass killed by grizzly bear within Waterton Lake Park.

Populations and Seasonal Movements

Inasmuch as the writer was able to undertake only brief periods of winter work in the parks, when snow conditions made it possible to determine most easily local populations and movements, a large part of the data gathered relative to this subject comes from the Park wardens of Jasper and Banff National Parks.

There have been changes in the local distribution of wolves from one year to the next and as these throw some light upon the factors influencing movements the local situations will be given in detail.

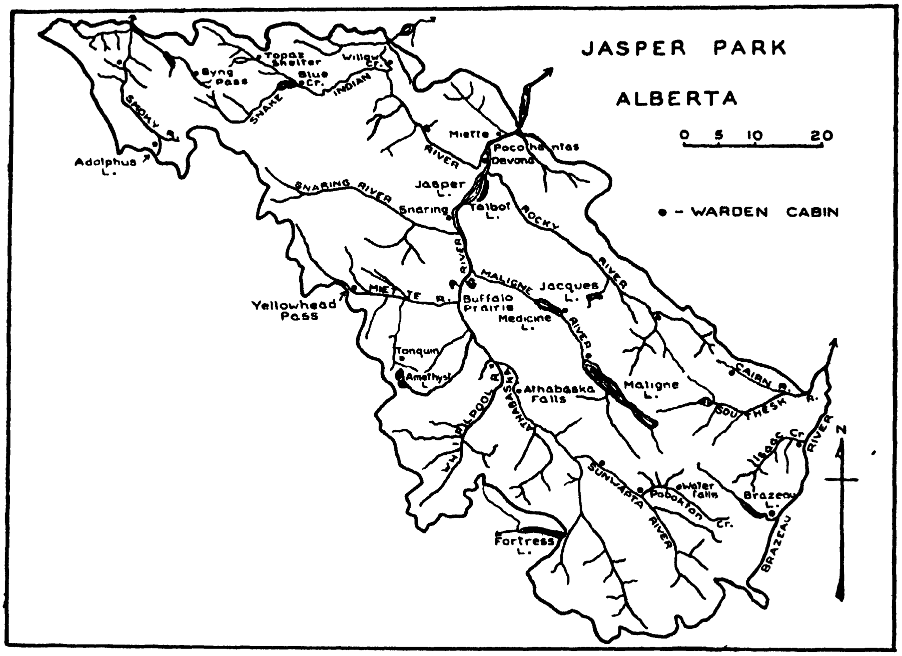

FIG. 1. *Jasper Park, Alta.*

In the valley of the Smoky River, Warden F. J. McGuire reported in 1943 that during the previous two winters, after the snow became crusted, there were three groups of wolves working the valley periodically. One consisted of seven animals, one of two, and one was a single individual. These wolves each came down the valley approximately once a month. The last wolf tracks seen in the Smoky Valley in 1943 were those of a pack of 13 or 14 that came up the Smoky and over on to the Moose River on Dec. 15. There were no wolves present during the late winter and spring. In the winter of 1944-45 the snow lay deep and soft over the entire valley until late March and the wolves remained outside the park until the month of April when a few entered, coming up river. In the winter of 1945-46 a band of five or six wolves came up the Smoky from outside the park in December. The only other wolves seen in that warden district during the winter were three in January 1946. After several unsuccessful attempts to kill moose they returned down river and left the park.

There is a small summer population of wolves in the Smoky Valley and its subsidiary stream valleys. In August, 1943, there were tracks of at least three different individuals on Byng Pass between the head of Twintree Creek and the head of the Snake Indian River. Two wolves worked the Byng Pass country (Fig. 4) during the summer of 1944 but produced no pups.

There is no information available on the region west of the Smoky Valley to the interprovincial boundary.

Wolves summer in the upper part of the Snake Indian Valley, including Blue Creek Valley, and stay in the autumn until snow conditions render travelling difficult. In 1942 they stayed until mid November and then withdrew down the valley. In 1943 Warden R. Jones found wolves working the upper Snake Indian and Blue Creek Valleys until almost Christmas time, when they moved down river; they did not return until early May. The largest band noted in the valley in the winter of 1942-43 consisted of 11 wolves. At different times during the early winter tracks of three were seen at Hoodoo Creek and again at Willow Creek. In this spring movement six wolves passed up the Snake Indian River to an undetermined destination beyond Hoodoo Cabin and were not seen again. There were no signs of recent wolf activity in the vicinity of Blue Creek in late May, 1944.

In August, 1943, wolves were heard howling near Blue Creek cabin and tracks of three individuals were seen along the trail to Topaz Lake. There were also tracks of three on Byng Pass, but whether these two occurrences represent the same individuals is not known. They are less than 15 miles apart. In October, 1943, Warden R. Jones saw a large pup wolf at Blue Lake at the head of Blue Creek.

At Willow Creek, in the Snake Indian Valley, there is a resident group of wolves that has numbered four or at the most five individuals constantly during the past three years. Apparently no pups have been produced by this group during this time. They apparently range a fairly small area from Rock Lake up Rock Creek to Mowitch Creek and along the flats bordering the Snake Indian, perhaps two or three miles beyond the confluence of Deer Creek with the River. The total area of their normal year round range apparently does not exceed 50 square miles.

The population of wolves wintering along the Athabaska Valley from Buffalo Prairie to Miette cabin has increased during the three years of this study. In the winter of 1942-43 nine wolves wintered on the benches adjacent to Devona (Fig. 6) and the mouth of the Snaring. These consisted of a group of four and a single animal and apparently two pairs though one of the pairs periodically joined the group of four.

A group of six seen on Pyramid Lake in the early spring was almost certainly the same group reported by Warden Bert White of Decoigne as moving down the Miette past his warden station at approximately monthly intervals. There was apparently another small pack concentrating its activities on Buffalo Prairie. In 1943, then, there were possibly 21 wolves wintering in or periodically entering the Athabaska Valley below Athabaska Falls, and these, with four at Willow Creek, four or five on the Brazeau, six or seven on the

Upper Athabaska and Whirlpool, and the 10 to 13 that moved up the Smoky in December, make up the probable total population of Jasper Park in the winter of 1942-43, a population of from 45 to 50 individuals.

During the summer of 1943 wolves apparently denned in the vicinity of Yellowhead Pass or Decoigne. During the last week in August four wolves were seen in close pursuit of a cow moose in this area; tracks were noted from time to time. Warden White of the Decoigne area during 1944 shot or snared nine wolves at Decoigne, including two pups taken in September of that year. None was resident there in the summers of 1944 and 1945 and the travelling band in the winter of 1944-45 was down to four individuals. Disease, as will be mentioned later, probably had some part in this reduction. In the summer of 1946 wolves were present at Decoigne and an adult female was shot.

In the winter of 1943-44 a band of four wolves worked the benches adjacent to Devona, two more ranged from Devona to Miette, and a single animal roamed the area generally. Six wolves left the valley in early May and moved, almost without pause, the 56 miles from Devona to Hoodoo Cabin and passed on up toward Byng Pass. The summer of 1946 first saw an established population remaining on the Athabaska Valley winter ranges. In May and June fresh wolf tracks were continually present on the Maligne horse range and in the valley of Vine Creek.

Warden Frank Wells in January saw tracks of two small packs, one of five and one of seven, on the Buffalo Prairie area (Fig. 5) under circumstances that led him to believe they were different bands. He also believed that the dozen or so wolves that ranged the upper Athabaska and Whirlpool areas that winter did not include the Buffalo Prairie packs. In January, four wolves came down Poboktan Creek and down the Banff–Jasper Highway as far as the Athabaska Falls cabin. At Decoigne wolves were active in October but they left during the latter part of the month and did not return until about Christmas time. About ten came through then but moved on and wolves were not again seen until Mar. 31 when from seven to nine entered the area. The pack split up and apparently settled in for the summer. It was at this time and during the succeeding eight months that the nine wolves were taken by Warden White. These Decoigne wolves are almost certainly part of the groups recorded as wintering on the Athabaska Valley.

The summarized count of wolves in Jasper Park in the winter of 1943-44 then would be: along the entire Athabaska Valley, approximately 30; Brazeau Valley, 4 or 5; Willow Creek, 4; or a minimum of 38. If we consider the 13 that came up the Smoky on Dec. 15 as the same as the group (estimated at about 10 animals) that came down the Miette a few days later—and there is good reason to take this view, as when last seen on the Smoky the pack was heading for Moose River, which in turn would take the pack down to Yellowhead Pass and on to the main wolf run through Decoigne—and if we regard this band as made up of animals not already tallied we can add this number, and also the four that came down Poboktan Creek in January and arrive at a maximum population figure of 55.

The winter of 1944-45 found the wolf population slightly smaller. As already mentioned, snow conditions in the Smoky Valley kept the wolves at lower elevations and outside the park. F. A. McGuire reported that a pack of six or seven was working the winter ranges just below the park boundary most of the winter.

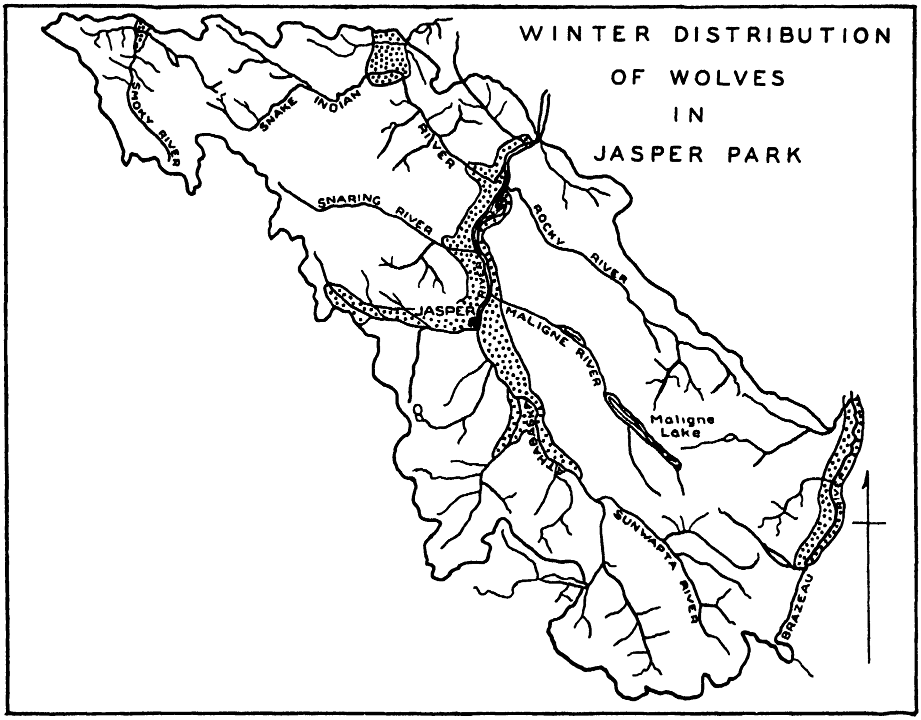

FIG. 2. *Winter distribution of wolves in Jasper National Park.*

Frank Wells found that a group of about seven ranged from Athabaska Falls to the Whirlpool River. This is doubtless the same pack that Warden George Camp reported as traversing the Whirlpool district at fairly regular intervals during the winter. Camp was sure of but five animals in the group. On each occasion that these wolves entered his area they came from the west and moved down the river as far as Moab and Otter Creeks, where they crossed over toward Geraldine Lakes and the Athabaska River above the falls.

On one or two occasions another pack of five or six came up the Athabaska from Buffalo Prairie, following the route of the highway to the falls area.

The presence of this group of five on Buffalo Prairie is confirmed by Frank Wells, who also suspected the presence of a pair running separately from the other five. There was another group of two or three in the Honeymoon Lake area.

The Decoigne area was visited periodically by four wolves; five ranged the Devona flats and benches, two lived in the Miette region, the same lone wolf present during the three previous years was still in the Devona–Miette area and it was probably this individual that was seen by Warden Brasnett and some others near Talbot Lake and the mouth of Rocky River. The Willow Creek band this winter numbered five and Warden J. St. Marie was able to confirm my previous estimates of the wolf population of the Brazeau River as five animals. It thus appears that the winter population in the Athabaska Valley in 1944-45 was about 23 wolves while the approximate population of Jasper Park was between 33 and 39 animals.

All these estimates are based upon the assumption that there are no wolves living through the winter in the area drained by the Snaring River. Nothing is known of the wildlife conditions of this region, but what has been reported of the terrain and its winter conditions leads to the assumption that wolves would probably be unable to winter in it.

Minimum and maximum population estimates for Jasper Park between 1942 and 1946 were:

1942-43	Minimum	45	Maximum	50
1943-44	"	38	"	55
1944-45	"	33	"	39
1945-46	"	38	"	48

If we assume that the maximum figures represent the true conditions, the density figure for Jasper National Park has been approximately one wolf for every 87 sq. miles. If the minimum figures are closer to actuality the density, based upon the area of summer range, was 111 sq. miles per wolf. At the time of maximum winter compression, however, this population is present on an area that averages approximately 10 sq. miles per wolf.

It is more difficult to estimate the population of wolves that from time to time enters the boundaries of Banff Park. Much of the park has been virtually without winter patrols and as a result there is less known of winter wildlife conditions in it than in any of the other Rocky Mountain Parks.

Only on the Saskatchewan and Panther Rivers was there evidence of a resident wolf population in 1943 (Fig. 3). In that year, also, wolves were present in summer on the Ranch and adjacent areas as well as on Snow Creek. The total of wolves periodically entering Banff Park that winter did not exceed 12 or 13 individuals. In 1944-45 evidence indicated that perhaps four wolves had been working in and out of the Park along the Red Deer and Panther Rivers. Five were seen at one time near Saskatchewan Crossing. To reach a total figure we should perhaps add the lone wolf reported to be in the Bow Valley and the three that spent a few hours in the Park on the eastern end of Lake Minnewanka. This would give a total of 13 again for the 1944-45 winter, or 10, neglecting the last trio. There may have been a slight increase in 1945 as the best available figures for the wolves entering Banff Park during the winter of 1945-46 indicate a minimum population of 12, a maximum possible of 18. This year saw wolves become established in the Clearwater Valley where they ranged into the Park throughout the year.

The early winter of 1946-47 saw the establishment of a pack of seven to nine wolves in the Bow Valley 12 miles west of Banff marking a new extension of range southward in this Park.

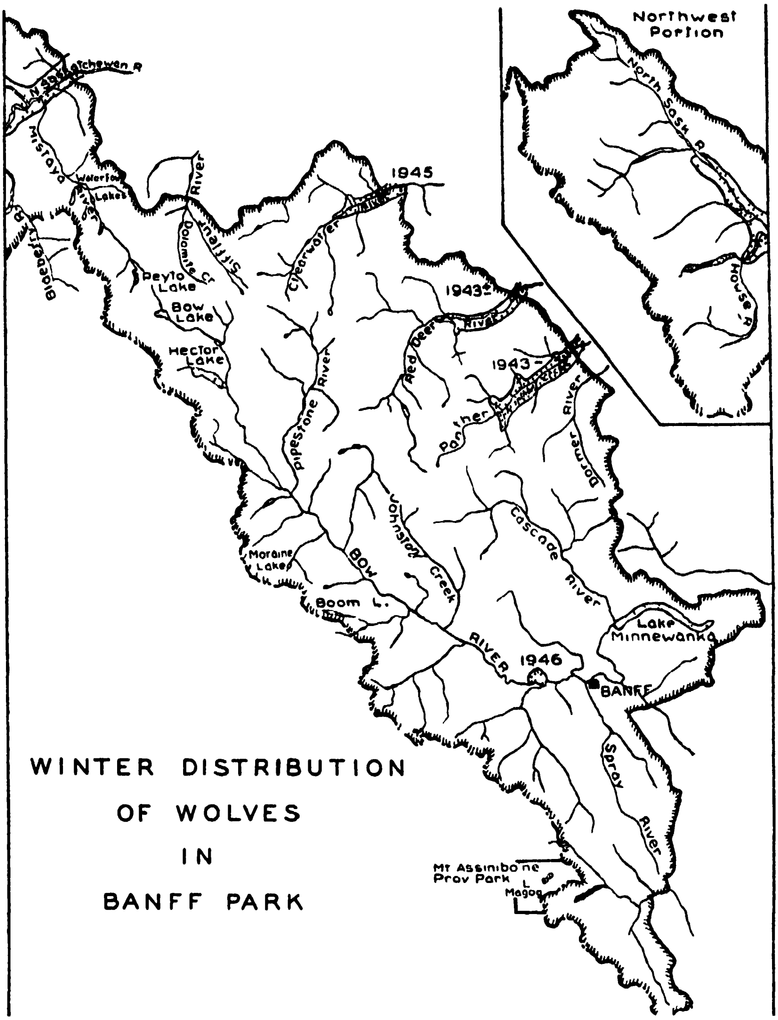

FIG. 3. *Winter distribution of wolves in Banff National Park. Dates indicate years in which resident populations became established.*

Movements

As has been indicated above, there is considerable variation in the amount of seasonal movement undertaken by various groups of wolves. During the summer months, when winter hunting packs are broken up and the animals hunt in ones and twos, there is evidence that certain wolves still live a roving existence. For instance in the Brazeau Valley in 1944 two wolves had preceded us all the way down the trail from Brazeau Lake Cabin to six miles or so below Isaac Creek, a total distance of some 22 miles. Sign along this trail, both then and in 1943, showed this to be a regular travel route.

Other groups of wolves are fairly sedentary during the summer. For instance in 1943 and again in 1944 there were two or three wolves almost constantly present on Byng Pass and up Blue Creek. The summer of 1944 saw a relatively sedentary group working about 10 miles of the upper Miette Valley adjacent to Decoigne. There are always wolves on Buffalo Prairie—in 1945 these consisted of at least three adults and the pups of one pair.

Certain of the wolf groups remain relatively sedentary even during the winter months. For example the Willow Creek group of four individuals in 1943 and 1944, five in 1945 is apparently constantly on an area of some 72 sq. miles.

As mentioned above, there are wolves present on Buffalo Prairie throughout the year, and, inasmuch as the area is hemmed in by roads on the north, west, and south and backed against the mountain on the east, it is possible to keep fairly close watch on the movement of wolves into and out of the area. There are transient bands there from time to time but the resident animals roam an area of approximately 55 sq. miles.

Other groups become transient during the winter. Possibly they are wolves that summered at altitudes unsuited for winter range, either because of snow depth or because of efflux of game. These groups apparently have a fairly circumscribed summer range and a separate winter range at considerable distance from it. The wolves from the Byng Pass and Blue Creek areas apparently move down into the Athabaska Valley in midwinter and remain there until late April or early May.

The summer and winter ranges of this band are some 60 to 70 miles apart.

The indications are that certain packs hunt over considerably larger circuits than those already mentioned. B. White at Decoigne has remarked upon almost monthly re-appearance of wolves in his area during the winter. The wolves almost always come through Yellowhead Pass from the west and proceed down the north bank of the Miette River, keeping to the road or to the more open forest on the benches above the road. They leave the river somewhere above Geikie and strike across the higher benches to the vicinity of Pyramid Lake. From there they drop down on to Henry House Flats (Fig. 8) through a narrow cut in the escarpment wall. Where they go to from here no one knows, but somehow they get back across the interprovincial boundary within the month and continue the beat. The circuit must be at least 70 miles in length.

There is one instance of a pack of 13 or so coming up the Smoky and over Moose Pass. A pack of almost the same reported size went through Yellowhead Pass in an easterly direction a few days later and there is good reason to believe that it was the same one. The latter pack had travelled a distance of some 70 or 80 miles at the very least and probably much more.

PLATE I

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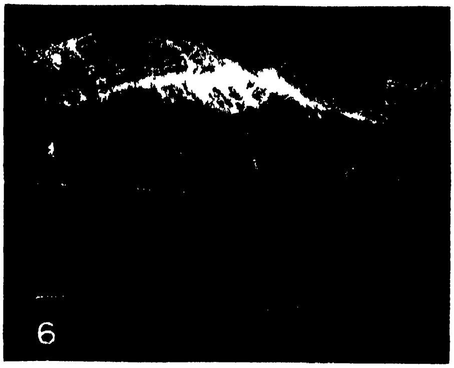
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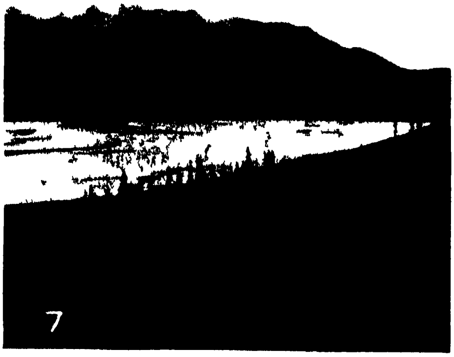
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FIG 4 *Summer range on Byng Pass, Jasper Park, Alta*

FIG 5 *Year round range on Buffalo Prairie, Jasper Park*

FIG 6 *Devona winter range from the south, treeless lower slopes constitute the most heavily populated game winter range in the Parks*

FIG 7 *Close study of Devona range showing heavy overgrazing and absence of escape terrain, April, 1943*

FIG. 8 *Henry House Flats winter range, Jasper Park, heavily stocked with elk and mule deer.*

FIG 9. *Terracing on Devona range by bighorn, elk, and mule deer.*

PLATE II

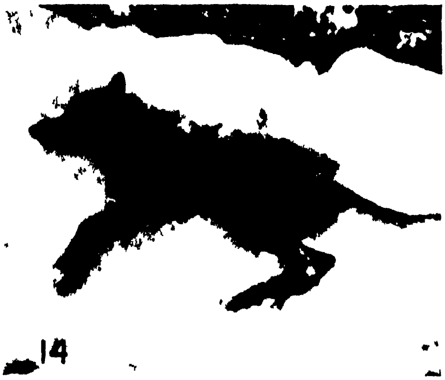

FIG. 10. *Shelter den under large boulder, Buffalo Prairie, Jasper Park.*
FIG. 11. *Den in beaver lodge, Buffalo Prairie, Jasper Park.*
FIG. 12. *Den on Buffalo Prairie, Jasper Park, occupied in 1945.*
FIG. 13. *Rock slide used as playground by pups from den illustrated in Fig. 12.*
FIG. 14. *Wolf with mange, Banff, Alta., January, 1947. Photo by U. LaCasse.*
FIG. 15. *Bighorn rams far from escape terrain, on Devona range, April, 1943.*

A similar monthly cycle of movement has been noted by George Camp in the Whirlpool Valley and by other wardens on other areas.

Population Controls

It will be obvious that the wolf population in the parks, inasmuch as it is little more than holding its own numerically must be suffering continual losses.

Destruction by man has taken a considerable toll of the Jasper wolves. During the four years of this study 24 wolves were shot and killed or seriously wounded by the Park wardens, 11 of them in 1944. This destruction was in furtherance of a recommendation contained in my 1943 report that limited control be applied, to the end that the population pressure within the Park not exceed that on the surrounding terrain. In Banff Park, during the same period, five adult wolves and two young pups were destroyed, while just outside the Park boundary the destruction of two dens yielded 11 pups and one adult.

As no studies have been undertaken on the provincial territory adjacent to the park it is not possible to quote figures of the approximate population of wolves there. Verbal reports from Mr. Rufe Neighbor of Entrance, Jack Hargreaves and George Wells of Jasper, and from other informed persons points to a somewhat higher concentration of wolves outside than inside the park. To this extent, then, the limited control applied by man as a supplement to natural population checks has served its purpose. Just how much influence the human control has had is problematical.

Several diseases have been reported from wolves in various parts of North America. Rabies was reported by Seton (11) and by others. Distemper has been reported by many writers on wolves particularly in the Arctic regions where domestic dogs are in widespread use in the wolf range. It may well be one of the most important diseases of this animal. Encephalitis has also been noted and proposed as an effective population control.

Sarcoptic mange has been the only disease positively identified in the wolves of Jasper and Banff Parks and it has not been at all widespread. In midwinter 1944-45, B. White of Decoigne shot an adult wolf with a serious case of mange and in September of the same year he saw another wolf that appeared to be mangy. An adult female shot at Devona by F. Burstrom in early 1946 and one of two seen on Buffalo Prairie, Jasper Park, on Dec. 18, 1946, were mangy. Coyotes in the same area exhibit a high incidence of infection with sarcoptic mange and my studies lead to the conclusion that it is the most important control upon coyote numbers. Many serious cases have been seen in adult animals but the most serious effect is upon the pups. Apparently a high proportion of the pups in both Jasper and Banff areas succumb to this disease.

In November 1946 Warden U. LaCasse of Banff Park found a shelter den 15 miles west of Banff. This was being used by a group of from seven to nine wolves. The badly decomposed carcass of a wolf lay at the entrance to the den but its condition made determination of cause of death impossible. Another member of this band seen, and later shot (Fig. 14), on the Hillsdale meadows, 12 miles west of Banff, was badly denuded of its fur as a result of mange.

On Jan. 3, 1947, biologist E. W. Pfeiffer and Warden U. LaCasse while trailing the Hillsdale pack came upon a recently dead, frozen yearling she-wolf. It had died in a shallow den under an adjacent rock and had been dragged out by the other wolves. This wolf had 40% to 50% of its body area mangy and was extremely emaciated, weighing just 37 lb. Upon autopsy it was found that the liver bore numerous yellowish lesions scattered through the tissue, each about 4 mm. in diameter. The blood vessels of the stomach were greatly distended and the stomach and intestine contained, besides some parasitic worms, only blood, this in large quantities. This wolf had died following an internal haemorrhage but the nature of the condition leading to this could not be determined.

I have examined only five wolves for parasites. Four contained the tapeworm, *Taenia hydatigena*, one of these also harboured *Toxocara canis*, and another, adults of the granular tapeworm, *Echinococcus granulosus*. This is the tapeworm the larvae of which form the hydatid cysts common in the lungs of elk and deer in the Jasper area. *Taenia krabbei* has been taken, as a larva, from caribou and again the wolf is the logical terminal host.

Breeding

Little first hand information on breeding behaviour has been obtained during this study. The wolves are most vocal in January and February and it is assumed that this urge to sing is here, as elsewhere, an accompaniment of the early phases of the reproductive cycle. Indications are that the young are born in May and June and as the gestation period is known to be about 60 days (9) mating must take place in March and perhaps early April.

Fierce fights sometimes take place during the mating season. John Shelford of Wistaria tells me of coming upon the site of such an encounter. For many yards the ground was strewn with fur and spattered with blood. The mangled remains of a large black male testified to the fate of the vanquished. Another case of physical combat was encountered by Park Warden Frank Camp and his companions in Jasper Park in January 1946. Coming on the site of a conflict as indicated by tracks and sign on the snow they determined that four wolves had attacked a fifth, and after a vicious fight the single animal had retreated wounded. They followed and shot the animal, which was then sent to me for examination. One foot was broken and there were many surface wounds in the skin of its neck and flanks. The canine tooth of one of its adversaries had punctured the hard palate and driven deep into the nasal passages.

Two litters were discovered in the study area in 1945. About June 7, 1945, Frank Wells found a den (Fig. 12) on Buffalo Prairie, Jasper Park. The young, apparently four in number, were active but still in the den. When the area was again visited, on June 15, the pups had left the den and were found to be hidden among the huge boulders of an adjacent rockslide (Fig. 13). Two were seen on this date and two others heard whining deeper in the slide. The pups apparently lived in and around this slide for some days as there were many scats in the vicinity and well-worn trails led here and there among the rocks or headed into the dim dry recesses deeper beneath the boulders.

In early June 1945 an Indian known as Silas discovered a wolf den on the banks of the Saskatchewan River two miles east of the Park boundary. In this instance there were five young in the den. These were dug out and destroyed.

In 1946 no dens were discovered in Jasper Park. The Buffalo Prairie den was deserted, though there were wolves in the vicinity.

Two dens were discovered in the Banff Park area in 1946. One of these was discovered by Warden Chas. Page on the bluffs overlooking the Panther River, some six miles below Windy Cabin. Mr. Page dug out this den in the last week of June and found four black pups in it. Two of these were destroyed, one eluded capture, and one was held captive until July 10, on which date we tagged and released it at the den site. The female and remaining cub were seen on the day we released the captive and there is little doubt that the family was successfully reunited.

The second den was discovered by William Winters on the bluffs overlooking the Clearwater River, five miles east (outside) of the Park boundary. This den yielded seven small pups and the female.

There are apparently many non-breeding individuals among the wolf population of the parks. Several groups upon which a careful check has been kept through three years have produced no young—or at least no young survived to join the winter hunting packs. The Willow Creek group has for three years consisted of from four to five individuals. The Brazeau River band has apparently consisted of from four to six animals constantly over the same period. The pair that wintered on the Miette sheep range from 1942 to 1945 was not accompanied by young.

The reason for this failure to reproduce is not known. It is known that female wolves do not breed until two years old and this will provide one source of non-breeding animals.

Some pups are lost to unknown causes. For example, the adult female shot at Decoigne on June 12, 1946, was given careful examination. The uterus and mammary glands gave positive evidence of this wolf having had a litter this year, but she had not been nursing for some time and had almost certainly lost her young.

Another possible explanation may be indicated by the sex distribution of the wolves destroyed in the Park during the four years. Of 25 for which record of sex has been available, 15 were males. The factors influencing sex proportions in wild animal populations are very imperfectly understood but anything that upsets the 50 : 50 ratio in a monogamous species such as the wolf will exert profound influence upon the reproductive potential of the population. It seems to me that this, together with losses to mange and to old age, probably in large measure explains the failure of the Rocky Mountain Park wolves to increase during the three year period of this study.

Pups have been noted in three parts of Jasper Park under circumstances that suggested the presence of dens in the vicinity. They are Decoigne, Buffalo Prairie, and Honeymoon Lake. There is probably another wolf den somewhere adjacent to Hardscrabble Pass at the head of Blue Creek, where a pup was seen by Hatter on June 17, 1945, or perhaps at some point further down Blue Creek, where a pup was shot by Warden Jones in October, 1946.

Dens

The Saskatchewan River den as described to me by its Indian discoverer was dug into the sandy marl of a knoll adjacent to the river. It had a depth of about 10 ft. but further details were unobtainable.

In mid April, 1945, Frank Wells found what appeared to be the beginnings of a den on Buffalo Prairie. Here an ancient beaver house, about 8 ft. in diameter, left dry by the breaking of the dam, had been opened at both ends (Fig. 11).

When it was examined by me on May 4 it showed signs of recent excavation, and there were fresh wolf tracks in the mounds of loose earth at the tunnel entrances. The burrows were 18 in. in diameter and approximately circular. This den was not used after our visit and the later discovery of an occupied den about a half mile away suggests that the beaver house site was never intended for such use.

The Buffalo Prairie den discovered by Frank Wells in early June 1945 was in an almost ideal location (Fig. 12). A number of huge boulders resting partly buried near the crest of a rocky ridge elevated some 60 or 70 ft. about the general level of the surrounding country and sparsely timbered, constituted the den site. The den itself was entered down a short slope between two large rocks and then a tunnel measuring 16 in. high by 18 in. wide had been excavated under the largest of the boulders. It led back about 7 ft. into a dry, slightly enlarged chamber that apparently served as the nest, although no bedding of any sort was present. A few feet away was another burrow under a large rock, apparently used at one time as a den but more recently as a shelter. Fifty feet away, on an aspen covered slope, a chamber (Fig. 10) large enough to accommodate two or three adult wolves in shelter and dryness had been dug under another large boulder.

A rock slide, composed of the debris from a 100 ft. cliff offered shelter and a playground for the pups among the large rocks heaped in disorder.

A fine clear spring emerged from the ground not 15 yd. from the den.

Remains of prey animals representing several years of habitation were strewn about the vicinity.

The Panther River den found by Warden Chas. Page in June 1946 was situated on the side of a small depression in the summit of a ridge bordering the river. The den was about 12 ft. in length and instead of penetrating straight into the hill it paralleled it after reaching a depth of about 5 ft.

A search of the area adjacent to this den led to the discovery of four other dens in fair state of repair and six others, much older and now caved in. All but one of these were near the crest of the 30 to 40 ft. bluff that borders the river course and had a broad view up and down the valley. Characteristically the dens had two entrances, a few feet apart and converging on the main tunnel some six feet from the entrance. These entrance tunnels were large enough to permit me to enter in a prone position.

At Hillsdale in Banff Park in January, 1947, a pack of five wolves was using a group of blown down pines, with their needles still on, as a shelter. Into this den they had carried quantities of elk hair, possibly attached to chunks of meat used for food.

Territorial Defense

Twice, in May and early June 1945, prior to his discovery of the Buffalo Prairie den, Park Warden Frank Wells had his dogs molested by wolves. On May 24 he, with his three dogs, two cougar hounds, and a black-and-white collie mongrel, were proceeding across country two or three miles from the den when three wolves began to circle the group, howling as they did so. Wells finally shot and wounded one wolf and, as his collie ran in, the wolf locked jaws with it, tearing out one of the dog's upper molars. The wolf escaped. A few days later, while he was riding through the area with his dogs, a large black wolf rushed from cover and seized the collie, first by the throat and then by the small of the back, inflicting deep wounds. Wells rushed to the rescue of the dog and the wolf left, then as he bent over the injured and apparently dead dog, he became aware that the wolf was closing in on the run. By shouting and waving his arms Wells checked the animal's rush while it was still a few feet distant and it then made off into the timber. The dog lived.

The circumstances do not seem to indicate that the wolf was knowingly attacking a man. It was aroused to a killing frenzy and the man was half hidden from its view and probably unrecognizable.

Relations with Other Carnivores

There has been little opportunity of gathering data on this aspect of wolf biology. Little sign of active hostility between wolves and coyotes has been seen, though the latter are normally present as scavengers at any meal the wolves provide for themselves. In December 1943 F. Burstrom at Devona found where four wolves had caught and killed a coyote and torn it to pieces. In the same region in August 1945 he saw five coyotes following a large black wolf. The wolf paid little attention to the coyotes except to threaten any that came too close. Near Jasper in December 1946 J. Hatter noted that coyotes were almost always present in the vicinity of the wolves. According to figures supplied me by men who have hunted both animals from an automobile on the ice of Ootsa Lake, B.C., the wolf at its best seldom attains a speed greater than 25 miles per hour while the top speed of a coyote is almost 10 miles an hour faster. This being the case the coyote is well equipped, under ordinary circumstances, to stay out of reach of the wolf.

Present indications are, then, that wolf–coyote interaction is largely beneficial to the coyote through carrion made available at kills not completely consumed by the wolves.

The grizzly bears of Jasper Park are, unlike those of other parts of the Rocky Mountain Parks, actively predaceous and subsist to an important degree upon elk and moose run down and killed by themselves. Grizzlies normally feed for several days upon such a kill and cover the remains beneath a pile of debris at the end of each meal. Where wolves are not present the bear frequently does not remain in the near vicinity of its kill between meals. However, in the autumn, if wolves are present and in hunting packs, the bears change their behaviour and bed down close to any kill they make. This behaviour has been noted by several wardens and evidence of it has been seen by myself. In three such instances the bear had made its bed close up against the partially eaten and buried carcass. Frank Wells, in the autumn of 1944, came upon one large grizzly as it lay spread-eagled upon the top of the buried carcass of a large bull elk.

Tracks have, in several instances, revealed where wolves had circled about a bear defending its kill but no attempt had been made to molest the animal.

In conversations with several guides that take hunting parties into the region north of Jasper Park several accounts have been heard of wolves molesting grizzlies; none of these was told to me by the viewers and they were accepted with reservations. One guide even went so far as to state his opinion that (in certain areas) the grizzlies are so constantly harried by wolves that they forsake the regions for others containing fewer wolves. No factual basis for such an opinion has been advanced by anyone.

Hunting Behaviour

Much has been written concerning the hunting behaviour of wolves in various parts of the country. The best accounts are those given by Murie (9) in his study of the wolves of Mount McKinley. Observations made during the present study confirm those of other authors. The usual winter hunting pack consists of from four to seven individuals, with five or six the most frequent numbers. Packs of 10 or a dozen have been reported once or twice in the Jasper area. The largest group recorded was believed to contain 14 individuals.

While travelling in the snow over ridges or other unpromising game country, or when merely travelling, the wolves in this area characteristically travel single file, the leader breaking trail and the others treading in the same tracks. Then, upon entering a swamp, swale, or thicket, the pack changes to the hunting order, which is line abreast. In this region, where much of the hunting is done in timbered country with restricted visibility, the line abreast hunting pattern enables the pack to sweep a wider band. When game is jumped the wolves are often in a position to outflank the animals and to effect speedy capture.

In the Hillsdale area of Banff Park, there is much abruptly rolling country with steep sidehills and sharp ridge tops. Here, in January 1947, with 18 in. of snow on the ground, the wolves were running the ridge tops until the elk were spotted on the slope below them. A quick downhill rush and simultaneous attack by several wolves was almost always successful in an immediate kill.

At Patricia Lake on Dec. 24, 1946, five wolves had jumped a three year old bull elk from its bed in a willow swale. They drew blood within 20 yd. and killed the beast before it had run 120 yd.

During the summer wolves frequently hunt alone or in smaller groups and a lone wolf is capable of pulling down the largest of the game animals at present occurring in the Rocky Mountains. Several instances of single wolves killing moose and elk were noted.

Several kills involving elk were examined in the spring of 1943 and 1944, and in the winter of 1945. Most of these had not been hamstrung nor had they been pulled down by the throat. In each instance the attack had been from the rear and side with the wolf seizing the flank at the point where the leg joins the abdomen. A wound here lets the viscera out, with the opening of large blood vessels and reasonably rapid death.

One adult cow elk pulled down near Decoigne in April .1943 was examined carefully by B. White who could find no wounds but a small tear in the left flank. The single wolf responsible for this kill had eaten a few pounds of flesh from the udder and upper leg and did not return to the carcass—possibly because of human interference.

An adult bull elk examined on Apr. 20, 1943, near Decoigne, had been killed by six wolves. After a short run this animal had been overtaken and pulled down. The nose, throat, and flanks were torn. A young bull elk examined on Apr. 23, 1943, at Mile 38 on the Banff–Jasper Highway had likewise been seized by the throat.

No instance of hamstringing has yet been seen or reported to me.

Long pursuits are unusual in the Rocky Mountain region, probably because the varied terrain usually permits a quick termination of the chase one way or another. The longest chase we have noted was at Pyramid Lake in December, 1945. Here a small pack ran a yearling elk about a mile and a half before killing. Blood showed on the snow for the last quarter mile.

Contrary to the opinion of many people the wolf is by no means always successful in capturing the object of its chase. As already stated, the wolf's top speed under optimum conditions is only in the vicinity of 25 miles per hour. I have clocked running speeds of moose at 27 miles per hour, elk at 28 and 29, a buck mule deer running its best on rough ground alongside the highway reached a top speed of 30 miles an hour and averaged 28 miles per hour for about a quarter of a mile, by the end of which it was tiring quickly.

It will thus be seen that the ungulates can, over short distances at least, outrun a wolf. The defence technique most frequently resorted to by elk, moose, and mule deer when pursued by wolves is to make for the nearest water and take refuge in it. The wolves will not enter in pursuit. On several occasions single deer, elk, and moose have been seen making use of this defensive behaviour and Frank Wells tells me he has seen as many as 35 elk in a group standing in the Athabaska River.

A two year old cow moose, seen by picnickers on Aug. 24, 1943, was being closely pursued by four wolves. It beat the wolves to the Miette River, plunged in, and crossed over. The presence of people with a dog caused the wolves to drop the chase, but it is probable that the moose had already made good its escape. On the Smoky River in January 1946, Park Warden McGuire noted that three wolves had made several unsuccessful attempts to catch moose. Hampered by the deep snow, the wolves failed and later returned down river to areas where the snow was shallower.

In October, 1944, at Decoigne, a cow elk pursued by wolves made the river but died there as a result of flank wounds received during the chase. Warden White reports that the wolves made no effort to reach the carcass but left it to decompose. Frank Wells has noted three similar instances in his area.

Even the water refuge can be a not unmixed blessing under certain circumstances. In the late autumn, when water temperatures are close to the freezing point, deer and elk taking refuge in the open water of rivers not infrequently become so chilled that they collapse into the water and are drowned. No such instances were seen by me but several wardens gave first hand accounts of such events.

Occasionally an animal pursued by wolves will come to bay and beat off its pursuers. Such an instance was noted by F. Wells during the winter of 1944-45. Tracks revealed that a band of about seven wolves ran a cow elk down the bank of the Athabaska River above the falls. At the falls, where the river plunges deep into a short canyon, the elk came to bay on a narrow promontory that gave her protection at her flanks and back. The wolves after a bit of skirmishing gave up the chase and went in search of other prey.

In November 1945, Warden Aleck Nelles at Snaring Station followed three wolves and found where they had attacked a moose. The animal, instead of running, had turned at bay and, by striking with its forefeet, had successfully beaten off the wolves. Shortly after leaving the moose the wolves killed a deer.

In soft snow wolves are often at a disadvantage and under such circumstances Warden R. Jones has seen signs of many abortive chases after deer in the Willow Creek area.

When snow of a foot and a half or more in depth develops a heavy crust the advantage is reversed and wolves can kill deer practically at will.

Another hunting technique used in some instances is that of running game out onto the ice of frozen lakes and streams. Under slippery ice conditions ungulates are with difficulty able to run and not infrequently lose their footing and their lives.

An occasional animal, hotly pursued by wolves plunges to its death over a cliff or into a canyon. An elk and a moose died in this way in the winter of 1944-45 on the Red Pass road as a result of plunges into rock cuts on the old railroad right of way. Such instances are probably more accidental than purposeful.

When hunting small game, such as rabbits, the wolves usually move at a walk in what appear to be aimless wanderings. I saw no sign of an effort on the part of the wolves to track down the rabbit—rather the mode of hunting seemed to be designed to flush the animal by chance and to catch it in a swift rush.

I was not able to observe the technique used in hunting ground squirrels and marmots but it probably does not differ greatly from that used by coyotes in search of the same prey.

Food Consumption and Caching

It has been difficult to obtain information upon the frequence with which wolves make kills. The nomadic behaviour of the creatures makes it most difficult to determine this important element in wolf behaviour.

Under favourable local conditions, at Jasper, and at Hillsdale in Banff Park, in December 1946, and early in January 1947, biologist E. W. Pfeiffer, accompanied by Warden F. A. Bryant in Jasper, and U. LaCasse in Banff, was able to determine the frequence of kills for two separate packs of five or six wolves. Each of these groups was killing and consuming three elk in a two week period with indications that two small elk might be taken in a week. At this rate the wolves were accounting for from one to one and one-half elk per wolf per month in the midwinter months.

Wolves can at times consume large quantities of meat. At Hillsdale, Banff Park, on Jan. 7, 1947, five wolves killed and ate an entire calf elk. On or about Jan. 1 this pack had killed a cow elk and in two days had eaten the greater part of it.

Behaviour at this kill was characteristic of many others. After eating their fill the wolves retired a few yards to a knoll, there each animal dug a circular bed down to the pine needles, usually at the base of a tree. The bedding area was much padded down and strewn with faeces and indicated that the wolves had moved little until the greater part of the elk was eaten.

In March 1943 four wolves killed a mule deer doe near Devona and in about four hours stripped the greater part of the flesh from it.

In November 1943 Warden R. Jones came upon a young buck deer so recently killed as to be still warm and yet the five or six wolves in the pack had already removed most of the flesh.

On Nov. 1, 1942 Warden R. Jones came upon a group of half a dozen or so wolves feeding upon an adult buck mule deer. Back tracking the pack he came to the warm remains of a fawn. On the other hand there are the many times when wolves have travelled the trail for two or three days without any apparent kill.

In November 1945 three wolves at Snaring unsuccessfully attacked a moose and later killed and ate two mule deer and a calf elk all in five days.

F. Wells during a winter trip up the Snaring had occasion to follow the trail of a wolf pack of about six individuals. They killed two moose in three days eating only part of each.

The assumption that in each of the above instances the wolves ate all the flesh is probably erroneous. It is well known that wolves frequently will carry away large chunks of meat after they have fed to repletion. F. Wells, who has followed wolves with his dogs, informs me that quite frequently the dogs dig up meat cached by the wolves. Such caches were usually in some old mossy log. The meat was buried in shallow holes dug for the purpose. At other times it was merely buried in snow.

In November 1943 Warden R. Jones noted where five wolves had killed a deer near Topaz Lake. After feeding on the carcass they carried large chunks of meat to a nearby knoll where they buried them in the snow.

When hunting for den-bound pups the wolves have been known to carry home such large objects as the head and antlers of a seven point bull elk. At the Buffalo Prairie den on July 6, 1945, I counted the following animal remains—skulls of a yearling mule deer, a senile cow elk, two calf elk, an adult cow elk, a seven point bull with antlers on, part of another calf elk, and the right fore leg of an adult elk, the right hind leg and entire rib basket of a yearling elk, the left fore legs of a yearling, a two year old and an adult elk, both hind legs of another elk, the right hind leg of a yearling elk, the right front and left hind legs of a mule deer, and a beaver skull. When the den was visited two weeks earlier there were remains of a lamb sheep, consisting of almost the entire vertebral column.

At the Panther River den, when I examined it on July 10, 1946, there were few remains of prey animals. A beaver skull, parts of the fore limb of a deer and bones of a young cow elk, apparently killed right beside the den, were the remains present. The pups at this den were six weeks to two months old and had apparently been fed largely upon the flesh of large ungulates and upon mice. This pair probably stripped the flesh from the bones of their prey and brought home pieces of meat and hide. There was an adult cow elk kill about 300 yd. from the den and the cubs with their mother were feeding on this when they were first seen.

Wolves have the very dog-like habit of sometimes carrying an old bone with them. It is a fairly frequent experience among those who have followed the tracks of wolves, to have the wolf drop some old and meatless bone that it had apparently carried for several miles.

When feeding on a carcass wolves may or may not break and consume the long bones. If the prey is a young animal the leg bones and skull are usually eaten, except for the hardest parts, such as the teeth and the epiphyses of the long bones. The heavier bones of elk, moose, and adult bighorn rams are apparently beyond the power of the wolf to crack them. Even where game abounds one frequently finds quantities of dried leg skin and tendons in the scats. These indigestible items must be obtained from old and dehydrated carcasses.

Food Habits

Data upon the food habits of the wolves of Jasper and Banff Parks has been obtained from two main sources, the analysis of scats and the observation of kills definitely attributable to wolves.

The data obtained from the examination of kills is almost inevitably confined to the big game species. The smaller food items are consumed in their entirety. For this reason it is not possible to obtain an accurate picture of the diet of the wolves by this means alone.

Scat analysis has been widely used as a method of obtaining quantitative data upon the food habits of animals. Where the sample of scats obtained is unbiased as to source this method probably gives a fairly accurate representation of the actual diet. It is, however, most difficult to obtain a truly representative sample.

During the winter months the wolves often travel the park trails and leave scats where they are easily found. In the summer this is true to a much smaller degree. Particularly is this true of the wolves that repair to the alplands in the summer. Scats are left far and wide over the mountains and one finds few, even by diligent and painstaking search.

The winter scats so far obtained for the purpose of this study are probably fairly representative. The greater part of the winter scats are from the Snake Indian, Miette, and Panther river valleys, where the various big game species are present in approximately the same proportion that they are on the winter ranges in general throughout the park. Winter scats outnumber those representative of the summer season and inasmuch as the summer diet is apparently higher in the proportion of rodents eaten and correspondingly lower in game items it can be expected that additional data from the alpland regions particularly will change the proportion of rodents in the annual diet.

Half of the summer scats so far collected are from the Buffalo Prairie den. This is situated in one of the best beaver districts in Jasper Park, an area that is now deteriorating as a beaver habitat as a result of long-continued overpopulation, with consequent destruction of food trees. The beavers are, as a result, more vulnerable to predation than under average conditions.

In order to appraise the degree of selection undertaken by the wolves, or the ability of the various species of game to escape the assaults of wolves, it is

TABLE I

FOOD HABITS OF WOLVES AS REPRESENTED IN 420 SCATS FROM JASPER AND BANFF NATIONAL PARKS

Food items	Occurrences in winter scats		Occurrences in summer scats		Occurrences in annual diet	
	No.	%	No.	%	No.	%
Elk, *Cervus canadensis*	149	49	62	42	211	47
Mule deer, *Odocoileus hemionus*	46	15	20	14	66	15
Moose, *Alces americana*	26	9	6	4	32	7
Bighorn, *Ovis canadensis*	19	6	11	8	30	7
Caribou, *Rangifer arcticus*	14	5	1	0.5	15	3
Goat, *Oreamnos americanus*	2	0.5	0	0	2	0.5
Horse, *Equus caballus*	2	0.5	0	0	2	0.5
Total ungulates	258	85	100	69	358	80
Snowshoe hare, *Lepus americanus*	30	10	2	1	32	7
Beaver, *Castor canadensis*	5	1.5	25	17	30	7
Porcupine, *Erethizon dorsatum*	5	1.5	1	0.5	6	1
Ground squirrel, *Citellus columbianus*	0	0	6	4	6	1
Marmot, *Marmota caligata*	0	0	1	0 5	1	Trace
Vole, *Microtus* sp.	3	1	7	5	10	2
Coyote, *Canis latrans*	0	0	1	0 5	1	Trace
Deer mouse, *Peromyscus maniculatus*	0	0	1	0.5	1	Trace
Mantled ground squirrel, *Citellus lateralis*	0	0	1	0.5	1	Trace
Total rodents, etc.	43	14	45	32	88	18
Berries	1	Trace	1	0.5	2	0.5
Grass and sedge	2	0 5	—	—	2	0.5
Total item occurrences	304		146		450	

pertinent to attempt to determine the relative abundance of each species on the wolf range.

The only basis for this comparison lies in the total of animals seen on the general areas from which the wolf scats were collected. No special effort was made to find any one particular species and it is thought that the figures approximate the relative abundance of the six species of big game available to the wolves in Jasper Park except in the case of bighorn, which, by reason of their superior visibility, are possibly somewhat biased on this count.

In 1941 and 1943 game seen was as follows: Bighorn, 2251; elk, 1721; mule deer, 595; goat, 458; moose, 156; caribou, 36. The relative abundance of these species calculated from the above figures is given in Table II.

It will be noted that in several respects contribution to the annual diet is not strictly related to relative abundance. In comparison with the number of kills recorded, deer apparently contribute to the diet in greater proportion than they are represented in the population. For example, while only about one-third as many deer as elk were seen, deer kills were about half as numerous as elk kills.

While elk, deer, and moose appear in the diet in fairly close relation to their relative abundance it is apparent that sheep and goat are in another category.

TABLE II

CLASSIFICATION OF WOLF KILLS IN JASPER AND BANFF PARKS AND COMPARISON OF SCAT ANALYSIS AND RELATIVE ABUNDANCE OF GAME SPECIES

Species	Number of kills	Percentage of total kills	Percentage of item in scats containing game	Relative abundance of prey species, %*
Elk, *Cervus canadensis*	64	54	59	33
·Deer, *Odocoileus hemionus*	27	23	18	11
Moose, *Alces americana*	14	12	9	4
Bighorn, *Ovis canadensis*	10	9	9	42
Caribou, *Rangifer arcticus*	2	2	4	1
Goat, *Oreamnos americanus*	1	1	Trace	9
	118			

* *Based upon total counts of game seen during 1941 (1) and 1943 (2).*

They are apparently better equipped to escape the wolves than are the fores game species—or are hunted to less degree under the ecological conditions obtaining.

On the basis of present data the game species comprise 80% of the annual diet, while another 18% is supplied by the rodents. These percentages are similar to those determined by Murie (9) for the annual diet of the Mount McKinley Park wolves. In that instance game comprised 69% and rodents 27%.

On a seasonal basis the winter diet in the Rocky Mountains consisted of game 85% and rodents 14% whereas the summer diet was game 69%, rodents 32%. This reflects the greater availability of rodents during the summer, when the hibernatory species are active and the beavers are leaving their pond retreats in search of food.

Berries and grass have taken an unimportant part in the scats so far examined.

If the percentage composition of that part of the diet consisting of big game (358 items) is compared with the proportion of the various species of big game in the 118 wolf kills recorded there is remarkably close correlation indicated. This comparison is made on Table II. It will be noted that the various species occur in the same order of importance in both bodies of data and that the individual percentages are of corresponding orders of magnitude in most instances. This correspondence serves to reinforce the probability that the general pattern of food preference is reliably indicated in the present data.

Discussion of Food Items

Elk, Cervus canadensis nelsoni

The wolf range in Jasper and Banff Park is heavily populated with elk. In Jasper Park it is not possible to make an accurate census of the total elk population but it is probably not less than 3000 head. The winter range is, much of it, heavily overpopulated. Range conditions have deteriorated drastically (Figs. 7, 8, 9) and the reproductive rate has dropped until in December 1944 the calves numbered only 18% of the cows of yearling age and over.

The greater bulk of an individual elk, from two to three times that of a mule deer, when taken with reference to percentage occurrence in the scats, indicates that on the basis of present scat data elk are apparently being subjected to smaller proportional loss than are the deer. It should be mentioned that several bands of wolves, notably those at Decoigne, Athabaska Falls, and the Whirlpool are, according to field observations of the district wardens, subsisting almost exclusively on elk. Deer do not winter on these wolf ranges. Very few scats from these places are included among the sample analysed. For this reason it is believed that the actual kill of elk and its proportionate contribution to the annual diet is greater than indicated by present data.

In summer scats calf elk is represented in equal proportion with adult remains, a fact that reflects a relatively high take of the calves.

Mule deer, Odocoileus hemionus hemionus

Deer remains appeared in 66 scats and represent 15% of the annual diet. In a comparison of the game items alone, deer represents 18% of the annual diet. Inasmuch as 595 mule deer were tallied as against 1720 elk, it is probable that deer were one-third as abundant as elk in the wolf environment. On the other hand, on the basis of kills found, these two were contributing closer to the ratio of 1 to 2. It must be remembered also that one deer probably appears in no more than half as many scats as an elk. The inference is, then, that deer have been selectively hunted by the wolves.

It is not possible to estimate the total population of mule deer in Jasper Park, but in certain parts of the winter range the density is high. For example, several counts on the Devona winter ranges in May, 1943 and 1944, have yielded population figures of from 60 to 68 deer per sq. mile. In the vicinity of Jasper and of Henry House the winter population is estimated at 17 per sq. mile, whereas the marginal winter range at Willow Creek in May 1944, had five deer per sq. mile.

Another important factor involved in the preying of wolves upon deer is the physical condition of the deer. The seriously overgrazed winter ranges of the Athabaska Valley of Jasper Park do not offer an adequate supply of the most preferred deer food-plants, and as a result the fawns have difficulty in maintaining their condition and many die. Even so there are indications that predator pressure is not removing all the seriously weakened animals. In April and May of each year I have found several senile deer so weakened from parasite infestation and malnutrition that they were incapable of running. These animals would certainly have been eliminated had the predator pressure been heavy.

Moose, Alces americana americana

Moose remains appeared in 32 scats, 7% of the total sample. Thus on the basis of the scat analysis as also on the basis of the numbers of known wolf kills moose constitutes the third item in importance in the annual diet, while it is fifth in abundance in the total game population. This is in contrast with conditions reported from the Mount McKinley district of Alaska, where wolves seldom bothered moose.

Summer occurrences of moose are all those of moose calf remains. While it is certain that some calves are killed by the wolves, it should be borne in mind that the losses of moose calves to accidents are perhaps higher than similar losses in any other local species of big game. Carrion may well make up a fair part of the calf moose item. More than half of the occurrences of moose in winter scats also consist of calf remains.

Bighorn, Ovis canadensis canadensis

In view of the abundance of bighorn in the park and their apparent vulnerability to predation the small number of scats containing this animal is surprising.

On the Devona ranges large bands of bighorn graze upon the open grassy benches far removed from any terrain offering natural protection (Fig. 15). The behaviour of these sheep certainly leaves one with the impression that wolves occupy an unimportant place in their lives. Few wolf kills have been found on this area, though wolves have been present every year.

Most of the other sheep ranges of the park offer good grazing closely adjacent to rocky bluffs and cliffs where sheep could escape from wolves. On Dec. 26, 1944, James Hatter, assisting in this study, hiked from Devona to the Miette winter ranges. Sheep were much in evidence grazing on the grassy benches. The following morning no sheep were to be seen on these grasslands, they had all taken to the rocks. Hatter found a pair of wolves prowling in the vicinity. In the same area in May 1945 wolves were still present and the remains of two sheep may have represented successful hunting on their part. Though I searched the area thoroughly only these two sheep kills were discovered, along with the remains of one elk and a deer. The local sheep population varies from 100 or so to four or five times that number, in response to snow conditions at higher elevations.

It is noteworthy that the sheep on this range were wilder than on any other.

On Buffalo Prairie, where one occupied den was located and where wolves ranged through the year, the bighorn were ranging the low forested ridges (Fig. 5), feeding on the open, westfacing sidehills but crossing through the timbered valleys from one ridge to the next. As sheep range it is of a very low order with sparse food supply and few natural protective features save scattered small cliffs and rockslides and yet the sheep are apparently holding their own there. Their reproductive success is, however, lower than that of any other band. In December 1944 this flock was found to have a proportion of lambs to ewes of 10%, while the other flocks of the Athabaska Valley averaged 45%. The part played in this by the wolves is unknown. Coyotes were present in some numbers and mountain lions killed an occasional sheep.

Between Dec. 21 and 31, 1945, J. Hatter studied a group of five grey wolves working the northern end of Buffalo Prairie. A band of 10 bighorn (two rams, six ewes, two lambs) were frequenting Old Fort Point, a place offering excellent escape terrain close to a small feeding area. The wolves worked the point at about three-day intervals covering it thoroughly at each visit. Repeated census of the sheep revealed almost certainly that one of the lambs was taken during the 10 days.

In November 1944, F. Wells found an old ram that had been killed by wolves as it was crossing from Buffalo Prairie to the west side of the Athabaska River. Caught out on the timbered flats it must have fallen an easy victim. Remains of a lamb were found at the Buffalo Prairie den by Wells on his first visit to it. In other flocks near Jasper sheep were dying of old age during the winter. Two such senile rams came down from the slopes of the Palisades to die on Henry House Flats. This is an area regularly traversed by wolves.

The evidence seems to indicate that the Jasper Park wolves seldom hunt sheep, that the animals taken are those from time to time surprised away from escape facilities. However, the condition on the Buffalo Prairie area with regard to sheep is that the population is kept to a point of bare survival. From the National Park standpoint this is unimportant but from a game production standpoint this marginal sheep terrain might be made to produce a shootable surplus in the absence of wolves while in their presence it probably could not.

Caribou, Rangifer arcticus montanus

Little has been learned regarding the circumstances surrounding wolf predation upon caribou in the mountainous terrain of Jasper Park. Warden R. Jones of the Blue Creek area has seen evidence that wolves were pursuing caribou while these animals were wintering in the forested country between Blue Creek and Willow Creek. It is from this area that the scats containing caribou remains came. This animal occurred in 15 scats representing 3% of the annual diet.

Goat, Oreamnos americanus columbae

The preferred terrain of goats seems to render them almost immune to attack by wolves. One kill discovered by Warden F. Burstrom in December 1945 was a young animal caught crossing from one range to another. By frequenting the goat licks in the summer time wolves could quite easily find opportunity of attacking goats. At such locations, as, for instance, the Shalebank lick on the Snake Indian River, large numbers of goats pass through dense timber for more than two miles while going and coming between their mountain-top feeding grounds and the lick. Bears occasionally kill goats at this and other similar licks.

Snowshoe hare, Lepus americanus americanus

The snowshoe hare, or rabbit, is quite common locally in the Athabaska Valley but there has been no irruptive increase since 1915. Coyotes hunt the animals as an important part of their diet (5). In the Devona–Miette area, where rabbits are as common as they are anywhere in the park, 32 scats taken in 1944-46 contained this animal.

Beaver, Castor canadensis canadensis

In certain areas beavers are an important dietary item. For instance 25 out of 60 of the pup scats at the Buffalo Prairie den consisted entirely of beaver remains. Here beaver exceeded in importance deer and elk combined.

In 1930 the Athabaska Valley of Jasper Park was superlative beaver range and bore a very heavy population despite the abundance of predatory animals. The removal of trees by these beavers at the time when the elk and moose were eliminating all seedling trees has had the inevitable result of rendering large areas of the park unsuitable for beaver.

As stated above, Buffalo Prairie has for a long time been noted for the density of its beaver population. The many streams that wind between the rugged hills and ridges are a succession of old beaver meadows and dams in various stages of disrepair. There are still several active colonies, but the aspens, the favourite beaver food, have been killed to such an extent that certain beaver colonies are subsisting on jack pine. Others are wandering many yards from the ponds in search of food. These circumstances render the beavers easy prey to wolves, coyotes, and bears. There is little doubt that wolves and other predators are effective in reducing a beaver population that has eaten itself out. In this area the beavers have worked progressively further and further up small streams, so that when the last food is eaten they cannot find safe passage to a large river or lake. Many of the streams here are so small that they offer little protection to migrating beaver.

I have seen no evidence that predators can prevent a beaver population from increasing until it is so large that safely available food becomes inadequate.

Porcupine, Erethizon dorsatum nigrescens

Six scats contained porcupine remains, including some heavy quills. It is not known how wolves manage to kill porcupines without themselves receiving crippling loads of quills. Murie (9) describes an ingenious technique used by a husky dog in killing porcupines. It consisted essentially of seizing the animal by the nose and killing it with a bite through the head. Wolves apparently skin out the porcupine by opening it from the ventral surface.

Ground squirrel, Citellus columbianus columbianus

These animals are hunted by wolves to a certain extent during the summer months. At lower elevations these animals come out of hibernation in late April and some remain active above timber line until early September. During this time they figure importantly in the diet of coyotes. So far few wolf scats have been picked up on areas frequented by ground squirrels and it is possible that the 2% dietary item provided by this animal would be increased by further study.

Marmot, Marmota caligata oxytona

Marmots have not been common, during this study, on the parts of the alplands frequented by wolves and they are apparently not much hunted under existing circumstances. Just one scat contained marmot remains.

Mice

The microtines have been scarce during the period covered by this study, but, even so, coyote scats have contained their remains in appreciable numbers. Wolves apparently do not bother to hunt such small fry. Mouse remains occurred in seven summer and three winter scats.

Culling Effects

It will be understood that it is often most difficult to determine from the remains of a wolf kill whether or not the animal eaten had been in good or poor physical condition. Age class can frequently be determined and serve as indicative of presumed vitality and sometimes the remains may be fresh enough to permit determination of nutritional condition by means of an examination of the bone marrow.

It is inevitable, however, that the circumstances preceding the deaths of many wolf-killed animals remain unknown.

In an attempt to determine to what extent the wolves were preying upon cull classes rather than upon animals in the prime of life, all kills for which age or condition or both were determinable have been classified into three categories, namely, animals under one year, animals over one year but not senile or otherwise ailing or injured, and animals senile, diseased, or injured. In these groups are included all dead animals found, no matter what the cause of death. Because of the easily destructible nature of the skeleton, mule deer did not lend themselves to this classification; fawns were easily recognized but my figures for the other two age groups were biased by the number that had to be discarded as unclassifiable.

Elk and bighorn are particularly suitable for treatment and an examination of the data for these species reveals some interesting facts. Of 66 elk carcasses examined, 20 fell into the very young group, 29 into the mature category, and 17 were diseased or senile. In this species, then, it appears that almost as many animals are dying in the prime years of life as in the other two age groups combined. Actually, deaths during the first year greatly outnumber those in any other age group but the young animals are almost completely consumed by predators or scavengers and consequently leave few or no remains to be discovered. It is incontrovertible, however, that the mature category is contributing to a greater degree than would be expected if the losses were confined largely to the cull classes.

It should be mentioned here that the elk of Jasper Park are quite extensively infested with the lungworm *Dictyocaulus viviparus* and with the hydatid cysts of *Echinococcus granulosus*. The former is probably unimportant in mature, otherwise healthy elk, but the latter is known to have a readily discernible effect on the physical condition of infested hosts. The pulmonary situation of these cysts in elk might be expected to interfere still further with the ability of the infested animals to avoid wolf pursuit.

A certain proportion of the prime age group appearing as kills may owe its presence to this disease, which is not detectable from examination of the remains of a kill.

It has been shown elsewhere that the survival of calves is no greater on the wolf-free than upon the wolf-inhabited areas. There is thus no evidence that the wolves are selectively removing the young of elk.

The situation with regard to predation upon bighorn sheep is different. In this species the majority of the remains discovered have been in the old or ailing group and it has been shown elsewhere that the percentile losses of sheep during the first year are lower than for almost any other species. From this it can be deduced, either that wolves play so unimportant a role in the life equation of sheep that their kills do not alter the normal loss pattern appreciably, or that wolf predation upon sheep is, in large measure, on the cull classes. Field evidence favours the latter interpretation.

It is pertinent to emphasize that quite apart from the culling effect, it has been abundantly shown, by Errington (4) and others, that predation upon excess populations is relatively high. At current population levels perhaps half the elk in Jasper Park, and but little less in Banff Park, are surplus population.

Influence of Wolves on Sex Ratios and on Survival of Young Game

The successful analysis of predator–prey interaction is predicated largely upon a thorough understanding of the food habits of the predator and ecological circumstances that permit a direct approach to the problem of determining the net influence of the predator upon the prey. The presence of wolves over most of Jasper Park and their virtual absence from most of Banff, Kootenay, and Yoho Parks, while the other predatory species are fairly evenly distributed, offers such an opportunity of assessing the net effect of wolf predation upon big game stocks.

Data for use in this study were obtained by extensive vital statistics tallies of game herds in all parts of the Rocky Mountain Parks from 1943 to 1946. The totals available are much lower than the total counts of game seen because only those counts have been selected in which certain identification of the various sex and age groups was possible. These data were then segregated into groups based upon the presence or absence of wolves in the areas. The results are set forth in Table III.

TABLE III

COMPARISON OF SEX RATIOS IN ADULT POPULATION AND SURVIVAL OF YOUNG ON WOLF-INHABITED AND WOLF-FREE AREAS

Species	Wolf-inhabited areas						Wolf-free areas					
	Males		Females		Yearlings		Males		Females		Yearlings	
	No.	%	No.	%	No.	%*	No.	%	No.	%	No.	%
Elk	189	25	556	75	99	18	319	21	1168	79	296	25
Moose	105	64	60	36	13	22	102	60	69	40	16	23
Deer	152	34	291	66	90	31	78	36	140	64	35	25
Sheep	365	44	468	56	163	35	273	45	344	55	102	30

* *Expressed as a percentage of females over one year of age.*

It will be apparent from these data that there is a very close correspondence in the figures derived from the wolf-inhabited and the wolf-free areas. The survival of young elk to yearling age on the areas without wolves appears to be higher than it is on the areas hunted by wolves (25% as against 18%). However, figures taken from the spring census of elk in the Bow Valley made by H. U. Green in 1945 reveal that in this wolf-free area in an aggregate count of 1029 elk the percentage ratio of yearlings to females over one year was 19. This is virtually identical with the percentage on the wolf run areas of Jasper Park.

The slightly higher survival of fawn deer and lamb sheep to yearling age on the wolf-inhabited areas may or may not be significant, but it is difficult to avoid the conclusion that under the circumstances prevailing at present in the Rocky Mountains, circumstances that feature overcapacity game stock and badly depleted ranges, the wolves are not the critical factor influencing the survival of the young deer, sheep, moose, and elk to yearling age. Other studies I have undertaken in these parks (2 and 3) have made it most obvious that the critical limiting factor is range conditions. The comparative figures from wolf-inhabited and wolf-free areas reveal that at the present population levels of wolves and ungulates the net effect of wolves on game herd productivity in Banff and Jasper Parks is inconsequential. It is apparent, then, that evidence points to the wolf as being of lesser importance to the survival of game in the parks than are the welfare factors, of which the most important is food supply adequate in amount and in composition.

The present overcapacity game herds, inhabiting many parts of the Rocky Mountain parks, developed largely since 1930, at a time when predator control varied from none at all to light, with traps used infrequently, and poison strictly prohibited at all times.

There is thus further evidence here that, under certain circumstances, predators are powerless to prevent game irruptions. Under existing circumstances the predators present, coyote, wolf, fox, lynx, wolverine, mountain lion, grizzly, and black bear, together are not taking the annual net increment to the game herds, nor even removing the cull group, a large part of which becomes carrion following death from disease, parasitism, or malnutrition.

Summary

This study deals with a wolf population with a density in Jasper Park of one wolf to between 87 and 111 sq. miles of potential range, which is reduced to about 10 sq. miles per wolf during late winter, by contraction of available range. The range is heavily populated with big game, including bighorn sheep, mountain goat, elk, moose, caribou, and mule deer, so heavily populated, in fact, that the cumulative use of the restricted winter ranges has engendered a serious depletion of the browse plants and grasses to the end that the reproductive success of much of the game is now very low. The winter range of the wolves supports a mixed game population of between 30 and 40 head per sq. mile, as counted in the spring, or roughly 300 to 400 surviving head of game per wolf.

The study area includes 7000 sq. miles of mountainous terrain in the Rocky Mountains, including extensive areas on both the east and west slopes.

Wolves are unevenly distributed over this immense area to a degree that leaves almost half the area without a wolf population, although game of all types is present in abundance in the wolf-free areas and the lesser carnivores such as coyote, mountain lion, and wolverine are fairly evenly distributed.

During the four years occupied by this study there was no appreciable change in the wolf population. Failure to breed, disharmonious proportion of the sexes and death from mange are believed to be mainly responsible, though man has removed some wolves.

The annual diet of wolves in Jasper and Banff National Parks has consisted of 80% big game, with elk alone contributing 47%. Mule deer contribute another 15%. It is particularly significant that although bighorn sheep were in great abundance they were very little hunted by the wolves. The wolves of the Rocky Mountain Parks thus demonstrate a marked proclivity toward the elk as a food animal and take elk in preference to sheep even when both are equally available and both are in excess of the carrying capacity of the ranges.

Eighteen per cent of the annual diet consisted of rodents, of which snowshoe hare and beaver were the two most important.

The wolf range in Jasper Park was excellent beaver range 15 years ago but the very high concentration of beaver that developed has removed the food trees from almost all readily available areas. Because of the action of elk and moose in killing all reproduction of such trees within two or three years of its establishment, new food trees are not appearing. Under such circumstances many of the remaining beavers are highly vulnerable to predation.

An examination of elk, and bighorn found dead from all causes, including wolves, reveals that the elk population is suffering a relatively heavy loss in the prime age classes. The inference is made that wolves are not exerting a noticeable culling influence in their predation upon elk, so far as the term applies to the elimination of the weak. It is pointed out that in view of the overstocking of winter ranges as much as 50% of the current elk population can be considered as surplus and theoretically highly liable to predation. Losses to the sheep population, on the other hand, are confined largely to the young and old or diseased groups.

Vital statistics for game herds living with and without wolf pressure reveal that there is no discernible significant difference in the survival of young, or in the sex ratios within the two groups.

It is concluded that under existing circumstances the wolves are not detrimental to the park game herds, that their influence is definitely secondary, in the survival of game, to the welfare factors, of which the absence of sufficient suitable winter forage is the most important.

Any drastic reduction in the present elk herds, a reduction that is urgently needed, may quite well disturb the present balance and careful watch should be kept to the end that any major change in the position of the wolf relative to the other species of big game will be detected quickly.

Should the wolf turn to heavy predation upon mule deer, caribou, or moose, it may become necessary to attempt more intensive control.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 25, SEC. D. DECEMBER, 1947 NUMBER 6

A COMPARATIVE STUDY OF THE ATLANTIC SALMON, *SALMO SALAR* LINNAEUS, AND THE LAKE SALMON, *SALMO SALAR SEBAGO* (GIRARD)[1]

BY D. G. WILDER[2]

Abstract

Twenty-six body measurements and counts were made on the majority of 381 specimens of lake and Atlantic salmon. Adult lake and Atlantic salmon generally differ in coloration, spotting, and flesh color. Evidence is presented that indicates that these differences are not inherent but result from differences in the environment and diet. In the parr and in the spawning adult stages no consistent differences in body measurements could be demonstrated. Non-spawning adult lake salmon tend to have larger head parts and longer fins than fresh-run Atlantic salmon grilse. These differences are associated with a slower growth rate, maturation at smaller sizes, and a more pronounced retention of the secondary sexual characteristics in the lake salmon. The average counts for certain meristic structures tend to increase with increase in length. When lake and Atlantic salmon of approximately the same size were compared no consistent differences in these meristic characters could be demonstrated. Lake and Atlantic salmon may be inherently different with respect to migratory behavior but the evidence available to date fails to establish such a difference.

Introduction

Certain lakes in eastern Canada and in the northeastern part of the United States support populations of fish that are usually referred to as landlocked, lake, or sebago salmon. In the Lake St. John region of Quebec, the corresponding fish is known as the ouananiche. The close affinity of these fish to the Atlantic salmon (*Salmo salar*) has long been recognized but their exact systematic status has been a question of much discussion and speculation.

The lake salmon was first described by Girard (1) who distinguished it from the Atlantic salmon on the basis of general shape, head size, and spotting. Although his description was apparently based on only two specimens from Sebago Lake, Maine, he believed the fish to be sufficiently distinct to warrant the specific name *Salmo sebago.* Jordan and Evermann (3) considered both the sebago salmon and the ouananiche of Quebec to be subspecies of the Atlantic salmon listing them, respectively, as *Salmo salar sebago* and *Salmo*

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[1] *Manuscript received July 25, 1947.*

Contribution from The Department of Biology, University of Toronto, and the Atlantic Biological Station, St. Andrews, N.B. Based on a thesis submitted to the Graduate School, University of Toronto, 1940, in partial fulfilment of the requirements for the degree of Master of Arts, with financial assistance from the National Research Council of Canada.

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[The October issue of Section D (Can. J. Research, D, 25 : 135-174. 1947) was issued November 4, 1947.]

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salar ouananiche. Kendall (4), however, felt that the lake salmon was distinct from the Atlantic salmon and restored the specific name of *Salmo sebago*.

The most striking way in which lake salmon differ from Atlantic salmon is that they fail to migrate to salt water even though in most cases they are not truly landlocked or prevented from descending to the sea by any physical barrier. In addition to this behavior difference various workers have pointed out differences in size attained, in coloration, and in body proportions. In the present study several populations of lake and Atlantic salmon have been compared and an attempt has been made to evaluate the observed differences in the light of known environmental effects.

I wish to express my appreciation to Prof. J. R. Dymond who suggested this problem and under whose supervision the work was carried out. Numerous suggestions by Dr. A. G. Huntsman and Mr. H. C. White proved extremely helpful.

Specimens Available for Study

The summer of 1939 was spent at Grand (Shubenacadie) Lake, N.S., which supports a population of lake salmon. The Fish Culture Branch of the Department of Fisheries operates a rearing station for these lake salmon at the head of the lake. Specimens of these rearing pond fish were collected and examined throughout the summer, salmon parr were seined from several rivers in the vicinity, and some adult lake salmon were obtained from Grand Lake. During the summers of 1941 and 1942 observations were made on the Atlantic salmon of Moser River, Halifax Co., N.S.

In addition to the specimens obtained in the field the lake and sea salmon in the collection of the Royal Ontario Museum of Zoology were examined. Unfortunately the purity of the lake stocks is open to serious question. Extensive fish culture operations have been carried on in connection with the lake salmon and in the past both Grand Lake, N.S., and Chamcook Lake, N.B., or their tributary waters have been heavily stocked with young Atlantic salmon. At Grand Lake, however, all fish have free access to the sea through the Shubenacadie River and the rearing pond stock is obtained from wild adult fish that have voluntarily remained in the lake. At Chamcook Lake, of recent years at least, the migration of planted stock to or from the sea is prevented by an iron fence closing the outlet. Some of the Chamcook specimens, particularly those collected in 1938, may well be the progeny of Atlantic salmon parents. The locality, the number of specimens examined, and their condition at the time of capture are indicated in the following list.

Lake salmon

Grand Lake, N.S.

68♂, 84♀:—Yearling parr to adults reared entirely in artificial ponds. Collected from June to August 1939. Larger fish mature but not ripe when collected.

2♂, 9♀:—Three-year-old rearing pond stock released in spring of 1939, recaptured June 15 to July 6, 1939, immature.

3♂, *6*♀:—Adult wild stock, obtained from anglers' catches from June to August 1939, not ripe.

5♂, *5*♀:—Spent wild stock adults, stripped for hatchery purposes during November 1939, killed for examination Feb. 2, 1940.

1♂, *6*♀:—Adult ouananiche four years old. Eggs obtained from Lake St. John, Que., and reared in Grand Lake rearing ponds. Collected June 21 to Aug. 24, 1939, not ripe.

Rawdon River, N.S.

21♂, *4*♀:—Yearling and two-year old parr seined Sept. 9 to 18, 1939. Majority of males maturing.

Beaver River, N.S.

7♂, *2*♀:—Yearling and two-year old parr, seined Sept. 9, 1939.

Chamcook Lake, N.B.

8♂, *4*♀:—Yearling parr collected May 20, 1925.

14♂, *8*♀:—Spent adults collected May 20, 1925.

8♂, *4*♀:—Ripe adults collected Nov. 16, 1938.

Lake St. John, Que.

3♂, *2*♀:—Ripening adults collected Sept. 21, 1927.

Atlantic salmon

Benery brook, N.S.

8♂, *8*♀:—Yearling parr collected Aug. 11, 1939. Some of larger males maturing.

Sackville River, N.S.

10♂, *7*♀:—Yearling and two-year-old parr collected Sept. 6, 1939. Larger males maturing.

Smith brook, N.S.

11♂, *8*♀:—Yearling, two-year-old, and three-year-old parr collected July 22, 1939.

S. W. Margaree, N.S.

9 not sexed:—Yearling and two-year-old parr. Collected July 31, 1936.

Mersey River, N.S.

5♂, *4*♀:—Smolts collected May 15, 1931.

Salmon Lake, Que.

4 not sexed:—Atlantic salmon planted October 1936, collected Sept. 6, 1938.

Moser River, N.S.

5♂, *4*♀:—Fresh-run grilse collected July 11 to Aug. 7, 1941.

6 not sexed:—Large fresh-run salmon collected June 12 to July 3, 1941.

Blanc Sablon River, Que.

2♂, *5*♀:—Partially mended grilse kelts, spawned during fall of 1938, collected May 1 to June 8, 1939.

St. John River, N.B.

5 ♂:—Fresh-run grilse collected June to July 1934.

1 ♀:—Large spawning salmon collected Nov. 5, 1932.

Sackville River, N.S.

1 ♂, *1* ♀:—Spent grilse, collected Nov. 6, 1939.

Southampton River, N.B.

3 ♀:—Large spawning salmon collected November 1934.

Methods

Many of the specimens collected during the summer of 1939 were examined shortly after collection while still in fresh condition. The remainder were preserved in 5% formalin and examined during the winter of 1939-40. Since the museum specimens collected from 1925 to 1939 varied considerably in their state of preservation when examined in 1939, no attempt has been made to correct for the effect of preservation. Such measurements as body depth, body width, and eye diameter are particularly unreliable in specimens that have been preserved for a long time.

The various characters that were measured or counted are indicated below. Abbreviations that are used elsewhere in this paper are shown in parentheses. All measurements and counts were made on the left side of the fish, the measurements being recorded to the nearest millimetre.

Standard length (SL) was measured from the tip of the snout to the end of the vertebral column.

Head length (HL) was measured from the tip of the snout to the most posterior part of the operculum, exclusive of the opercular membrane.

Head depth (HD) was measured from the base of the occiput to the most ventral portion of the operculum.

Maxillary (Max) was measured from the tip of the snout to the posterior end of the maxillary.

Snout (Sn) was measured from the tip of the snout to the anterior border of the eyeball.

Interorbital (I) was measured from the mid-point of one supraorbital process to that of the opposite side.

Eye (E) is the anteroposterior diameter of the eyeball.

Body depth (BD) is the greatest body depth.

Body width (BW) is the greatest body width.

Caudal peduncle length (CPL) is the horizontal distance from a vertical line passing through the posterior insertion of the anal fin to the end of the vertebral column.

Caudal peduncle depth (CPD) is the least depth of the caudal peduncle.

Dorsal height (DH) and anal height (AH) were measured along the longest fin ray with the fin extended.

Dorsal base (DB) and anal base (AB) were measured along the body from the anterior insertion to the posterior insertion.

Pectoral length (PL), ventral length (VL), and adipose (A) were measured from the anterior insertion to the posterior tip.

Dorsal and anal fin rays are the total number of developed rays, including divided and undivided rays. One or two undeveloped rays normally present were omitted when calculating averages.

Pectoral and ventral fin rays are the total number of rays, including undeveloped rays if present.

Branchiostegals, pyloric caeca, and gill rakers are total counts, the raker count being made on the first gill arch.

Scale rows were counted along the lateral line from the posterior margin of the head to the end of the vertebral column.

Coloration

The general color pattern and the size and number of black spots are characteristics that are usually stressed in distinguishing lake and Atlantic salmon. The following descriptions are based on observations made in the field on fresh specimens.

During the summer months adult Grand Lake salmon are usually dark green on the dorsal surface of the head and body but variations from olive green to almost black are common. This greenish color fades towards the lateral line, the sides being a light silvery gray and the belly in most cases white. The pectoral fins are usually dark gray but may be tinged with yellow or olive green. The ventral and anal fins are usually lighter than the pectorals and in some cases are tinged with yellow. The caudal fin is almost invariably a dark uniform gray.

The majority of the lake salmon have from three to eight circular black or brown spots up to ¼ in. in diameter on the upper half of the operculum and cheek, but as many as 19 of these spots have been counted and in rare cases they are completely lacking. Posterior to the head the spots are smaller but more numerous extending below the lateral line on the anterior portion of the body. Usually there are 25 to 75 black spots above the lateral line and 10 to 25 below but in some specimens they are too numerous and indistinct to estimate while in others they are completely lacking. The dorsal fin that is the only spotted fin commonly has 10 to 20 black spots but in rare cases it is immaculate.

In the spawning season the lake salmon develop a pinkish band about ½ in. wide that extends along the lower side from the head to the base of the caudal fin. The pectoral, ventral, and anal fins, the outer branchiostegals and the posterior portion of the mandible also become tinged with this color. The posterior portion of the maxillary and the ventral half of the cheek and opercle usually turn a golden yellow while the caudal fin often becomes reddish brown. No sexual differences could be detected in this spawning coloration.

Atlantic salmon when caught in the sea are similar in color pattern to lake salmon but tend to be lighter in color, more silvery, and less heavily spotted. When they enter the rivers the blue green color of the dorsal surface quickly fades to a uniform gray. The sides of the head and body are very silvery and lightly spotted with small, often x-shaped black marks. The belly is white and the fins usually gray. These fish become darker as they remain in fresh water, the dorsal surface becoming almost black and the sides and belly turning gray. Individuals that have spent several months in the river are often referred to as 'black' salmon. Atlantic salmon kelts captured by H. C. White in the early spring of 1942 and retained in a small freshwater pond were very dark when examined by the writer during June and July. These fish had developed the numerous large black or brown spots on the head and body that are generally considered to be characteristic of lake salmon. Mr. White and the writer agreed that in general appearance these fish resembled lake salmon more closely than they did Atlantic salmon.

It is concluded from the above observations that the differences in coloration and marking commonly used to distinguish lake and Atlantic salmon are environmental effects and do not represent hereditary differences in the two groups of fish.

Flesh color

Atlantic salmon parr and smolts invariably have white or pale yellow flesh but in the sea the typical 'salmon-pink' flesh color is developed and the fresh-run grilse and larger salmon almost invariably have pink flesh. The lake salmon, however, retain the white or pale yellow flesh throughout life. This difference in flesh color has often been cited as proof of the distinctness of the two stocks.

The Atlantic salmon kelts retained by Mr. White (see above) were fed almost entirely on fresh gaspereaux (*Pomolobus pseudoharengus*) until their weight–length relationship equalled that of fresh-run salmon. These fish when examined by the writer during June and July were found to have white or pale yellow flesh.

Although it has not been demonstrated that lake salmon are capable of developing pink flesh it is believed that in both lake and Atlantic salmon the flesh color is dependent on diet.

Measurements

In comparing measurements of several samples of salmonid fish certain difficulties arise. Mottley (5) has shown that in Kamloops trout several characters grow at rates that differ from that of the fish as a whole. The eye, for example, throughout the size range studied grows at a much slower rate than the body with the result that large trout have relatively small eyes. Mottley has shown further that the relative growth rates of several characters are not constant throughout life. At the onset of sexual maturity such structures as the head, snout and maxillary, which have been growing at a relatively slower rate than the body, begin to grow at a relatively faster rate.

These observations have been confirmed by the writer for both the Atlantic salmon and for the speckled trout (*Salvelinus fontinalis*). White (6) has shown that slow growing Atlantic salmon parr tend to have larger heads and eyes than fast growing fish of the same size. In a homogeneous stock of speckled trout the writer (7) has demonstrated marked differences in the relative sizes of various body parts of trout reared from fertilization to hatching at different temperatures.

These differences in the relative sizes of body characters associated with absolute size, sexual maturity, rate of growth, and developmental temperatures render the assessment of differences in various stocks of salmon extremely difficult. To demonstrate inherent characteristics, the different stocks should be reared under identical conditions and specimens of the same size, age, sex, and state of maturity compared. This procedure, however, is not usually possible and in the present study an attempt has been made to evaluate the observed differences in the light of these environmental effects.

The various samples available for study are made up of fish that vary considerably in size range, state of sexual maturity, and in growth rate. Some of the samples are composed entirely of parr, other samples include only adult lake salmon, and others are made up of grilse and larger Atlantic salmon. Fortunately, however, a rather complete series of nonspawning Grand Lake fish ranging from 6 to 47 cm. in length is available and has been used as a standard for comparison with the other samples. The data for these Grand Lake fish were sorted according to length and sex and the average values shown in Table I calculated for each group of 10 fish. For the other samples in which the specimens extended over a sufficiently wide length range the individual measurements of each character were plotted against standard length on double logarithmic paper. Straight lines were fitted to these points by inspection and the size of each character corresponding to the average standard length of the sample was determined by interpolation. The grilse from the Moser, the Blanc Sablon, and the Saint John Rivers and the four Atlantic salmon from Salmon Lake extended over too short a length range to permit this method of analysis. With these four samples simple arithmetic averages were calculated. The values calculated for all samples other than the nonspawning Grand Lake fish are shown in Tables II and III. Many of the lake and Atlantic salmon male parr were maturing when collected during August and September but since no effect of this approaching maturity on the relative sizes of the body parts could be detected the sexes of the parr have not been considered separately.

Some of the male Atlantic salmon mature as parr at a standard length of 10 to 18 cm. After descending to the sea as smolts, these salmon grow at a much faster rate than the lake salmon, returning to the rivers about one year later as grilse at a standard length of 45 to 50 cm. The male lake salmon also mature as parr and the females start spawning at a standard length of about 25 cm. so that the both sexes spawn several times before they reach the size of Atlantic salmon grilse. This maturity at smaller sizes, associated with a

TABLE I

AVERAGE SIZES OF BODY PARTS OF NONSPAWNING GRAND LAKE SALMON. GROUPS OF 10 FISH, ALL MEASUREMENTS IN MILLIMETRES

	SL	HL	HD	Max	Sn	I	E	D	W	CPL	CPD	DH	DB	A	AH	AB	PL	VL
Males	84	21.8	12.3	10.0	5.9	6.3	6.2	18.6	12.2	14.0	7.7	13.8	12.0	6.4	11.1	7.0	17.9	12.8
	121	30.8	17.6	14.7	8.5	8.5	7.9	28.0	17.2	20.9	10.8	18.4	17.5	7.8	15.3	10.4	23.4	16.7
	160	38.7	22.3	17.6	10.1	11.5	9.4	33.5	20.8	28.0	12.5	20.3	21.1	9.8	16.4	12.7	26.8	19.1
	178	43.5	24.6	19.8	12.2	11.6	10.3	36.6	21.0	31.6	13.9	21.5	24.7	10.9	17.2	15.5	29.6	20.9
	191	46.3	26.6	22.0	12.7	13.0	10.5	40.9	23.8	34.8	15.1	22.8	25.7	11.9	18.7	15.3	31.2	22.6
	233	58.8	33.4	30.8	19.2	18.1	12.1	49.4	31.0	38.5	19.9	32.3	33.6	16.3	26.4	22.1	41.3	31.2
	288	70.0	40.8	35.1	22.1	23.2	14.0	61.9	39.2	49.3	23.4	34.8	38.8	19.4	30.7	25.3	46.9	33.8
	379*	89	51	49	31	28	17	70	44	68	27	44	50	27	37	36	57	44
Females	93	24.0	13.8	10.8	6.1	6.4	6.7	21.2	12.8	15.3	8.3	13.7	13.4	6.1	11.5	7.8	18.3	13.5
	128	32.5	18.2	15.1	8.9	8.8	8.3	26.8	16.9	22.0	10.7	19.1	18.8	7.9	15.6	10.8	24.1	17.8
	165	39.5	22.6	18.2	10.5	11.0	9.5	34.4	21.0	29.6	13.0	21.9	22.6	10.2	16.4	13.2	27.3	19.7
	186	44.4	25.1	20.6	12.1	12.3	10.4	37.6	22.7	33.0	14.7	22.2	25.7	11.0	18.4	15.6	29.9	21.6
	205	47.4	27.0	22.4	13.3	13.7	10.9	42.7	25.4	37.0	15.8	23.6	27.2	12.8	18.8	16.8	31.6	22.9
	248	56.6	33.4	27.0	15.9	17.5	12.6	50.9	31.8	43.7	20.0	28.3	33.5	16.2	24.3	21.9	38.9	29.0
	281	62.1	38.3	29.6	17.4	20.1	13.6	58.7	35.9	50.2	22.3	32.1	38.0	18.2	27.2	25.5	42.9	31.0
	302	66.2	40.0	32.1	19.2	21.4	14.3	60.3	38.1	55.2	23.5	33.7	38.6	19.5	28.2	26.3	44.5	32.3
	393	87.1	51.5	43.1	27.1	29.3	16.7	75.5	47.0	69.0	30.2	40.6	49.8	23.8	36.0	36.2	54.1	40.4

* *One specimen only.*

TABLE II

AVERAGE SIZES OF BODY PARTS OF LAKE SALMON. ALL MEASUREMENTS IN MILLIMETRES

	Sex	n	SL	HL	HD	Max	Sn	I	E	D	W	CPL	CPD	DH	DB	A	AH	AB	PL	VL
Chamcook Lake 1925	♂ and ♀	12	97	26	14	11	6 5	8 0	6 1	21	14	16	8 6	15	14	6 8	11	8.7	19	13
Beaver River	♂ and ♀	9	117	33	18	15	9 0	8 0	8 0	26	18	18	11	17	17	7 4	13	11	24	16
Rawdon River	♂ and ♀	25	132	32	19	15	9 2	9 1	7 8	29	19	22	12	20	18	8 7	14	12	25	17
Grand Lake planted stock	♂ and ♀	11	245	57	33	27	16	18	13	44	26	45	18	31	32	—	24	20	39	29
" " wild stock	♂	5	348	89	50	46	31	32	14	69	49	51	30	42	44	26	33	32	51	39
" " wild stock	♀	5	364	86	51	42	26	30	15	67	46	55	30	42	48	20	33	35	51	38
Chamcook Lake 1925	♂	14	380	91	53	44	27	32	13	90	41	63	33	50	49	24	39	34	58	44
" " 1925	♀	8	372	87	50	42	24	31	13	87	40	63	33	47	46	23	37	32	56	42
" " 1938	♂	8	263	68	36	34	22	22	12	54	29	41	23	38	36	19	30	24	47	35
" " 1938	♀	4	462	104	60	53	34	36	17	88	48	76	38	56	61	30	45	43	68	51
Lake St. John	♂	4	417	104	60	53	33	37	17	94	44	68	35	55	53	22	48	38	73	56
" "	♀	8	339	80	47	41	24	26	16	79	47	53	29	45	47	—	39	33	56	44

TABLE III

AVERAGE SIZES OF BODY PARTS OF ATLANTIC SALMON. ALL MEASUREMENTS IN MILLIMETRES

	Sex	n	SL	HL	HD	Max	Sn	I	E	D	W	CPL	CPD	DH	DB	A	AH	AB	PL	VL
Benery brook	♂ and ♀	16	92	24	14	11	6.2	6.0	6.7	20	14	15	8.8	14	12	7.0	11	7.5	20	13
Sackville River	♂ and ♀	17	103	27	15	12	7.0	6.4	7.4	22	15	17	9.3	16	14	7.3	12	9.6	22	15
Smith brook	♂ and ♀	19	110	28	17	13	7.6	6.8	7.6	25	18	17	11	17	16	8.4	13	10	24	16
S.W. Margaree River	?	9	131	31	19	13	8.2	8.7	7.5	32	23	22	13	17	20	9.6	14	13	23	16
Mersey River	♂ and ♀	9	204	47	26	20	10	14	10	41	21	38	17	28	23	11	22	17	35	25
Salmon Lake	?	4	316	64	30	35	18	22	12	60	39	60	24	34	36	18	29	24	44	32
Moser River	♂	5	478	100	58	49	34	37	17	107	59	86	37	50	58	29	45	40	65	49
" "	♀	4	472	90	54	44	30	34	15	102	59	—	37	48	59	29	42	42	59	46
Blanc Sablon River	♂	2	502	122	64	65	48	44	16	88	48	86	37	60	58	33	52	42	72	59
" " "	♀	5	462	99	60	48	32	37	15	84	47	79	35	56	54	26	48	41	66	50
Saint John River	♂	5	497	110	60	54	35	40	16	103	53	87	37	59	62	29	50	42	66	53
Sackville River	♂	1	471	108	64	58	41	41	15	95	50	72	40	51	59	32	49	39	66	53
" "	♀	1	487	106	62	53	35	37	15	107	50	77	39	52	57	32	47	44	69	51

slow growth rate between successive spawnings, results in the development of secondary sexual characteristics in relatively small lake salmon and a more pronounced retention of these characteristics throughout nonbreeding periods. The greater development of the secondary sexual characteristics in nonspawning Grand Lake salmon as compared to fresh-run Atlantic salmon grilse is clearly shown by the following measurements, all of which are in millimetres.

	Standard length	Head length		% Diff.	Maxillary		% Diff.	Snout		% Diff.
		♂	♀		♂	♀		♂	♀	
Moser River fresh-run grilse	480	100	92	8.7	50	45	11.1	34	31	9.7
Grand Lake nonspawning adults	350	83	75	10.7	48	37	24.7	30	23	30.4

With the lake salmon maturing at smaller sizes and retaining the secondary sexual characteristics to a greater degree it is virtually impossible, beyond the parr and smolt stages, to obtain specimens of lake and Atlantic salmon of the same size that have these characteristics developed to the same degree. Since, however, the changes associated with maturity are much less marked in the female salmon, these have been used in the preparation of Fig. 1. In this figure the data for certain characters of nonspawning Grand Lake female salmon are represented by straight lines. The data for the other samples of lake salmon are indicated by open circles and for the Atlantic salmon by closed circles. The particular sample to which each circle refers may be determined by reference to the average standard lengths in Tables II and III.

It may be seen from an inspection of this figure that the lake and Atlantic salmon do not differ consistently in any one character. Fresh-run grilse from the Moser River tend to have smaller head parts and shorter fins than the nonspawning Grand Lake stock. The spent or spawning grilse from the Blanc Sablon and Sackville Rivers, however, have larger head parts and longer fins than the nonspawning Grand Lake fish but are very similar to spent or spawning lake salmon from Grand Lake and Chamcook Lake.

Kendall (4) concluded that the most salient difference between the lake salmon and the Atlantic salmon is in the ratio of caudal peduncle depth to caudal peduncle length, the Atlantic salmon beyond the parr stage having more slender caudal peduncles. The present data do not support this conclusion since it may be seen in Fig. 1 that in the majority of cases the Atlantic salmon have caudal peduncles that are relatively shorter and deeper than the Grand Lake fish.

From the above data it is concluded that, in the parr stages, the lake salmon as a group do not differ significantly from the Atlantic salmon in any of the characters measured. The differences commonly observed in comparing adult nonspawning lake and Atlantic salmon are believed to result from a differential development and retention of the secondary sexual characteristics, which are associated with a slower growth rate in the lake salmon.

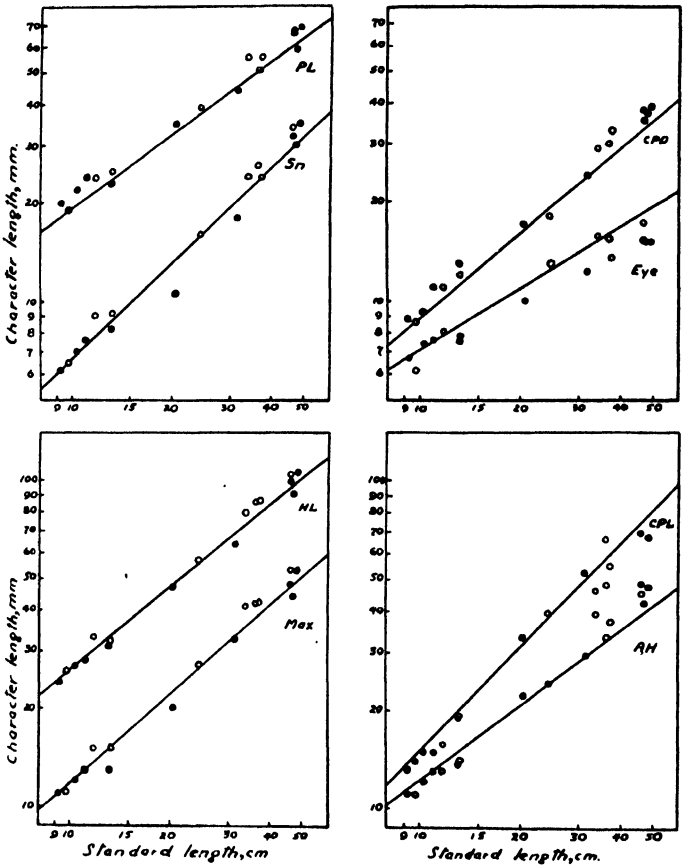

FIG. 1. *Relationship of character length and body length. Nonspawning Grand Lake salmon represented by straight lines, other samples of lake salmon by open circles, and Atlantic salmon by closed circles.*

Counts

Prior to comparing the average counts obtained for the various samples all the Grand Lake data were sorted according to size and sex. Since no sexual differences could be detected in these data, the sexes have been lumped in the following comparisons. The averages determined for the several size groups of Grand Lake salmon are indicated in Table IV where it is obvious that the average counts for certain characters tend to increase with size. This tendency when tested by the analysis of variance was found to be statistically significant (P less than .01) for all counts except the dorsal and anal fin rays. Because of this variation with size further comparisons have been restricted

TABLE IV

Average counts for different size groups of Grand Lake salmon, number of specimens in parentheses

Size group, cm.	Scale rows	Gill rakers	Pyloric caeca	Branchio-stegals	Pectoral rays	Dorsal rays	Anal rays
8-15	107.8 (16)	17.2 (26)	47.7 (7)	10.5 (28)	13.5 (39)	11.7 (39)	8.9 (42)
15-20	110.3 (35)	18.2 (35)	51.0 (20)	10.4 (51)	13.6 (45)	11.9 (29)	9.0 (51)
20-25	110.6 (19)	18.8 (22)	56.9 (21)	11.1 (25)	13.7 (25)	11.5 (19)	9.1 (25)
25-30	111.7 (32)	18.5 (32)	55.7 (32)	11.3 (33)	13.9 (33)	11.9 (22)	9.0 (33)
30 +	112.1 (27)	19.7 (29)	53.1 (28)	11.2 (28)	13.9 (29)	11.8 (25)	9.2 (29)

to samples of approximately the same average length. The 8 to 15 cm. group of Grand Lake salmon has been considered with the other samples of lake salmon parr and the 30 + cm. group with the other adult lake fish. The average values obtained for the parr and adult samples of both lake and Atlantic salmon are indicated in Tables V and VI. Since the ventral fin showed no variation in ray count, each specimen examined having a count of one undivided ray followed by eight divided rays, these data have not been included in the above tables.

TABLE V

Average counts for samples of lake and Atlantic salmon parr, number of specimens in parentheses

Population	Scale rows	Gill rakers	Pyloric caeca	Branchio-stegals	Pectoral rays	Dorsal rays	Anal rays
Lake salmon							
Grand Lake	107.8** (16)	17 2** (26)	47.7 (7)	10.5** (28)	13.5** (39)	11.7 (39)	8.9** (42)
Chamcook Lake	109.9** (12)	17.9 (11)		12.0** (12)	13.4** (12)	12.0 (12)	9.0 (12)
Rawdon River	112.6 (21)	18.4 (22)	52.3 (6)	11.2 (22)	13.8 (24)	11.6 (23)	8.9 (24)
Beaver River	112.0 (8)	18.1 (9)		11.9** (9)	13.9 (9)	12.4** (9)	8 9 (9)
All lake salmon parr	110.6* (57)	17.85* (68)	49.85 (13)	11.13 (71)	13.63**(84)	11.83 (83)	8.88**(87)
Atlantic salmon							
Benery brook	111 9 (15)	18.0 (16)		10.9 (16)	13.9 (16)	11.7 (16)	8.9 (15)
Sackville River	112.2 (17)	18.6 (17)		11.4 (15)	13.9 (17)	11.8 (17)	9.1 (17)
Smith brook	111.5 (18)	18.3 (19)		11.4 (19)	13.9 (19)	11.8 (19)	9.4 (19)
All Atlantic salmon parr	111.9 (50)	18.31 (52)		11.24 (50)	13.92 (52)	11.79 (52)	9.12 (51)

* *Differs from average for all Atlantic salmon parr at 5% level of significance.*
** *Differs from average for all Atlantic salmon parr at 1% level of significance.*

The average values for each sample of lake salmon have been compared with the over-all averages for the parr or adult Atlantic salmon, using the analysis of variance as a test of significance. Statistically significant differences are indicated in Tables V and VI by a single asterisk at the 5% level

TABLE VI

AVERAGE COUNTS FOR SAMPLES OF LAKE AND ATLANTIC SALMON ADULTS, NUMBER OF SPECIMENS IN PARENTHESES

Population	Scale rows	Gill rakers	Pyloric caeca	Branchio-stegals	Pectoral rays	Dorsal rays	Anal rays
Lake salmon							
Grand Lake	112.1** (27)	19.7** (29)	53.1* (28)	11.2 (28)	13.9 (29)	11.8* (25)	9.2 (29)
Chamcook Lake 1925	114.1 (22)	20.2** (22)	58.7 (6)	11.9**(22)	14.2 (22)	12.1** (22)	9.3 (22)
Chamcook Lake 1938	111.5** (12)	18.8 (12)	62.0* (8)	11.6 (12)	13.8 (12)	11.9* (10)	9.1 (12)
Lake St. John	114.0 (11)	19.1 (11)	63.3** (11)	11.2 (11)	14.4* (12)	11.7 (12)	9.6* (12)
All lake salmon	112.9** (72)	19.59**(74)	57.23 (53)	11.48 (73)	14.05 (75)	11.87**(69)	9.25 (75)
Atlantic salmon							
Salmon Lake	118.2 (4)	19 (1)		10.5 (4)	13.5 (4)	11.2 (4)	9.5 (4)
Moser River	117.1 (10)	18.3 (9)	55.0 (3)	11.3 (14)	13.9 (14)	11.6 (14)	9.1 (14)
Blanc Sablon River	111.3 (7)	19.3 (7)	60.6 (7)	11.6 (7)	13.9 (7)	11.1 (7)	8.7 (7)
Saint John River	117.5 (6)	19.0 (6)		11.0 (5)	14.2 (6)	11.2 (6)	9.2 (4)
Sackville River	113.0 (2)	19.0 (2)	55.0 (2)	11.0 (2)	14.0 (2)	12.0 (2)	9.5 (2)
Southampton River	114.7 (3)	18.3 (3)	53.3 (3)	11.3 (3)	14.3 (3)	11.7 (3)	9.0 (3)
All Atlantic salmon	115.6 (32)	18.78 (28)	57.27 (15)	11.20 (35)	13.94 (36)	11.42 (36)	9.09 (34).

* *Differs from average for all Atlantic salmon adults at 5% level of significance.*
** *Differs from average for all Atlantic salmon adults at 1% level of significance.*

of significance and by a double asterisk at the 1% level. In no case do the sample averages for lake salmon and the over-all averages for Atlantic salmon differ consistently. For each of the characters studied some samples of lake salmon averaged significantly lower than the Atlantic salmon whereas other samples either averaged higher or showed no significant differences. From these observations it is concluded that lake salmon as a group do not differ inherently from the Atlantic salmon in any of the meristic characters studied.

Migratory Behavior

Although nonspawning adult lake salmon generally differ from fresh-run Atlantic salmon grilse in having larger head parts, longer fins, and more numerous, larger spots these differences do not occur in the parr stages and it is believed that these are not inherent characteristics but result from environmental differences. There is, however, the possibility that lake and Atlantic salmon are inherently different with respect to migratory behavior. This possibility is being investigated by A. G. Huntsman at Grand Lake, N.S., where yearling lake and Atlantic salmon were planted together in June 1944 and 1945. To date (Huntsman (2)) it has not been possible to demonstrate any differences in the migratory behavior of these planted fish.

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THE GENETICS OF THE COLOUR PHASES OF THE RED FOX IN THE MACKENZIE RIVER LOCALITY[1]

By L. Butler[2]

Abstract

The red fox (*Vulpes fulva*) exists in the wild in three coat colour phases; red, cross, and silver or black. These three phases result from the action of one pair of alleles, the homozygotes being silver and red and the heterozygote being cross. At least two different mutations have occurred giving rise to the Canadian gene in eastern Canada and the Alaskan gene in western Canada. The mixing of these two mutant genes complicates the gene frequency analysis.

The proportions of the three colour phases are shown to vary with (1) locality, (2) state of population cycle, (3) population trend, (4) migration pressure. Of these factors the variations with locality and population trend are fairly satisfactorily explained by selection but the fluctuation of colour phase proportions with the population cycle is not. On the other hand all the facts can be explained by a migration theory, with or without selection. By migration, a mixture of native and migrant populations with different gene frequencies is obtained. Such migrations tend to be rhythmic since they are connected with the population cycle. In the year that migration took place the pelt returns reveal aberrant gene frequencies or colour phase ratios. In the following years the gene frequencies quickly approach equilibrium that may be at the premigration level or at a new one depending upon the success of the migrants in establishing themselves in the breeding population.

The marked diminution in the percentage silver and cross is due to the rapid population increase in an area of low frequency of the silver producing gene and the migration of this type into areas of higher frequency.

The red fox, *Vulpes fulva*, exists in three coat colour phases: silver, cross, and red. In a recent paper by Butler (2) the genetics and distribution of these colour phases across Canada were discussed, the data used for this purpose being the Hudson's Bay Company fur returns from 1915 to 1944. The gene frequency tests indicated that only in British Columbia were the ratios consistent with a single factor theory. Elsewhere they could be explained by the two factor hypothesis of Warwick and Hanson (1). The silver-producing allele *(a)* of the Alaskan factor is most abundant in the west and becomes decreasingly abundant in geographic progression as one moves eastward. The Canadian gene *(b)* has the opposite distribution. The data grouped in pentads by provinces revealed that the percentage silver had decreased steadily in most provinces. It was suggested that the reduction may be due to one or more of the following reasons:

1. The mixing of Canadian and Alaskan genes.
2. The northward migration of the southern type of fox with its lower frequency of silver-producing genes.
3. Selection against the silver type operating through differential fertility or survival of silver foxes in comparison to reds and crosses.

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[1] *Manuscript received August 6, 1947.*
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In order to throw more light on this subject the following paper deals with the fur returns of an isolated section for the periods 1860–1893 and 1915–1946.

The pelt data are collected chiefly from the Great Slave Lake region and the watersheds of the Mackenzie and its main tributary the Liard River (Figs. 1, 2). This country is bounded on the west by the Peel and Richardson mountain ranges, on the north by the Beaufort Sea, and in the east by the Arctic tundra. Migration, if it takes place, is therefore fairly well restricted except from the south. The fox species of this region according to Seton is *V. alascensis* with *V. abietorum* in the south and with *V. fulvus* coming in at the S. E. border. It is perfectly feasible to theorize that if there has been enough isolation to develop different coat and skull characters there should also be different colour phase ratios, and that any periodic migrations would mix these in various proportions.

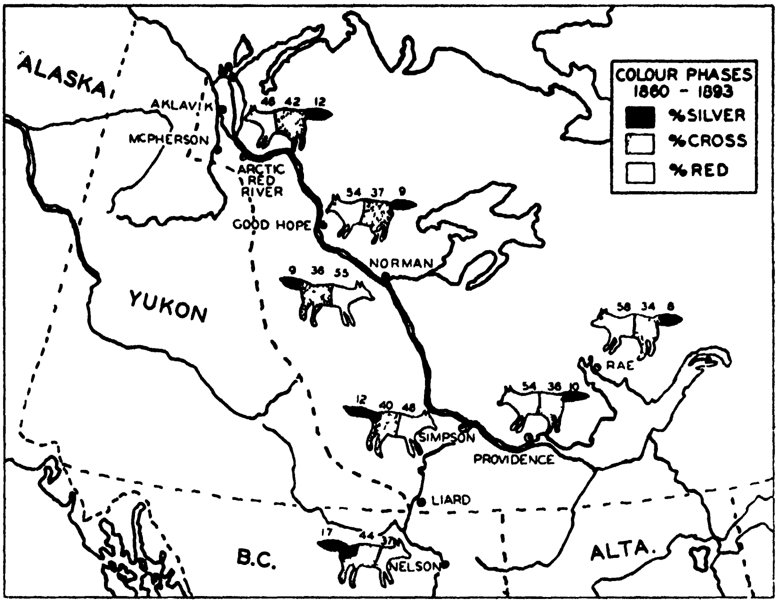

FIG. 1. *Geographic distribution of fox colour phases, 1860–1893.*

The Population Cycle

The yearly numbers of red fox are known to fluctuate rhythmically with high populations occurring over most of Canada every 8 to 10 years. Population cycles affect colour ratios (Butler (2, p. 48)) so population statistics are here presented for the information they may give on changes in gene frequency and possible migrational movements. The causes of the cycle may be food or weather factors as suggested by Elton *et al.*, or may arise from the interaction of environmental factors, but whatever their cause the solution of these genetic problems of populations leads us to consider them. Lack of coincidence in peaks, or peaks occurring in one locality and not in adjacent ones, provide valuable clues to migration and the possible mixing of races with different gene frequencies.

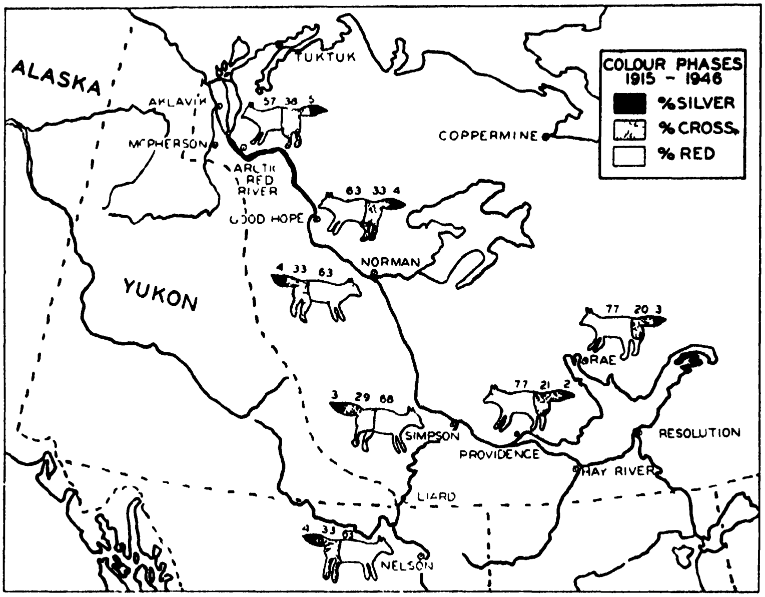

FIG. 2. *Geographic distribution of coat colours in Vulpes, 1915-46.*

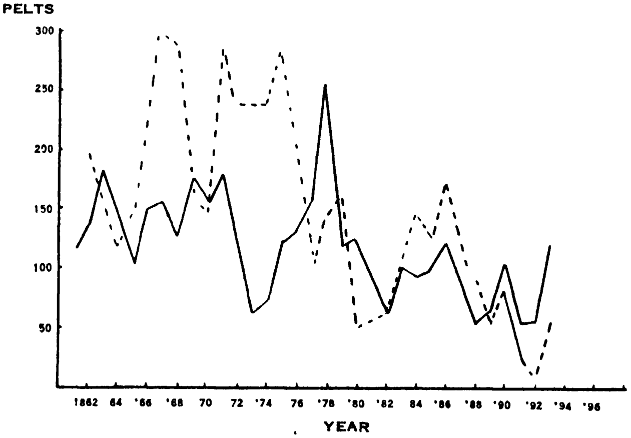

FIG. 3. *Total fox pelts taken each year at Forts Good Hope and Rae;* ——— *Good Hope,* - - *Fort Rae.*

The total number of fox pelts taken at two of the main collecting posts in the period 1860–1893 are plotted in Fig. 3. The data are plotted arithmetically to be consistent with the genetic data presented later. Examination of these early collection figures reveals no clear-cut 10-year cycle such as appears in the 1915–1946 data presented in Figs. 5 to 8. The chief characteristics of the collections are as follows.

Fort McPherson—violent fluctuations about a trend that increased up to 1875 and decreased thereafter. The main fluctuations correspond to the Arctic type of cycle with four-year peaks, which is characteristic of the white fox population of the delta region. In general, as can be seen below, the peak years of white and coloured fox coincide.

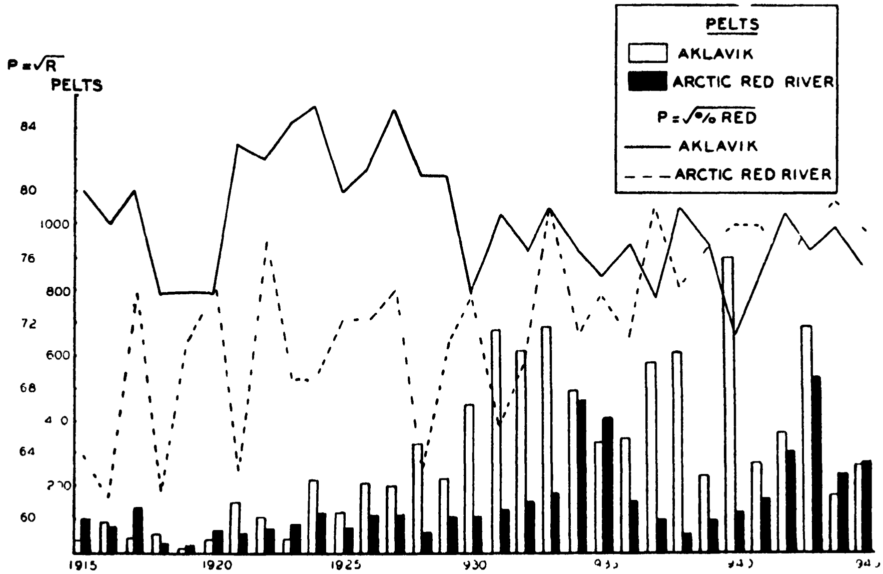

FIG 4 *Gene frequencies and population numbers, Aklavik and Arctic Red River*

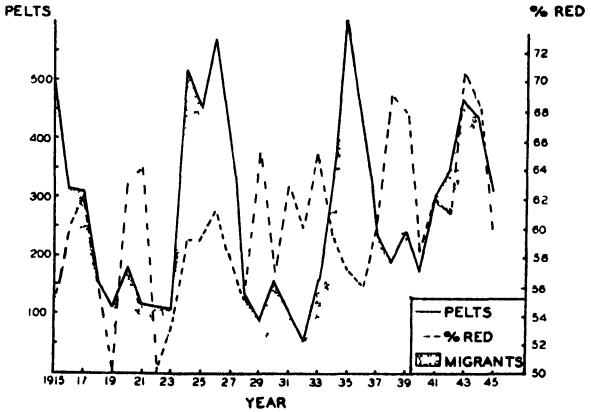

FIG. 5. *Number of migrant foxes in relation to percentage red and total pelts, Forts Good Hope and Norman.*

Fort Good Hope—a poorly defined 10-year cycle with some minor fluctuations that can be interpreted as being caused by the arctic cycle.

Fort Rae—a mixture of the arctic and 10-year cycle.

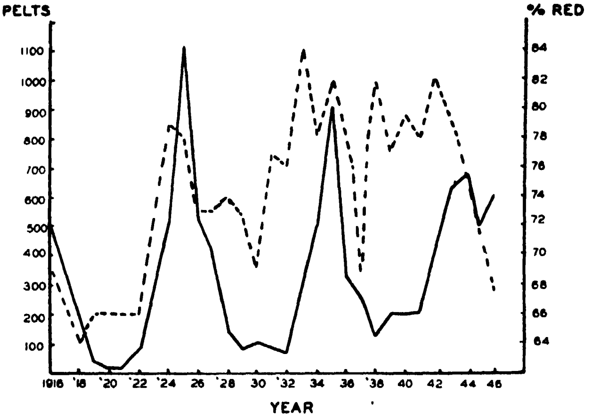

FIG. 6. *Total fox pelts taken and percentage of the red phase, Fort Providence and Hay River; ——— total pelts, - - - - % red.*

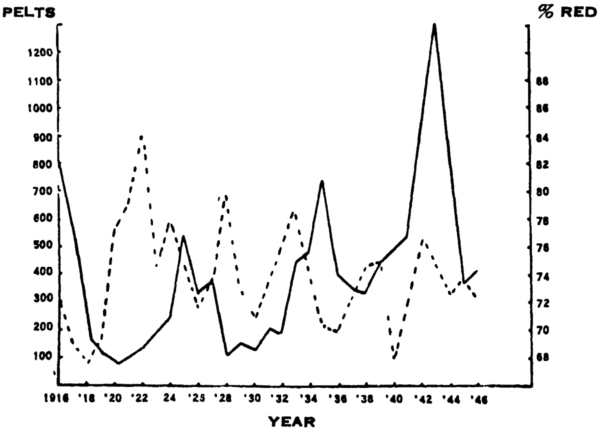

FIG. 7. *Total fox pelts taken and percentage of the red phase, Fort Resolution; ——— total pelts, - - - % red.*

The correspondence between peak years can be seen from the chart below.

PEAK YEARS

White Fox	1861		1864	*1868*		1872		1876	*1878*		1881	1884	1885		*1887*
Ft. McPherson	*1861*		1864	1868	*1871*		*1875*		*1878*		*1881*				
Ft. Rae				*1867–68*	*1871*		*1875*			1879				*1886*	
Ft. Good Hope		1863			1871				*1878*					1886	

The years shown in italics are the better defined peaks. The year 1871 was the only peak year at all points.

Turning next to the modern period, 1915–1946, we find that along with a general increase in numbers the pelt collections at Rae, Providence, and Good Hope are definitely of the 10-year cycle type (Figs. 4 to 6) with peaks in 1925, 1935, and 1944. In the first two peaks, Good Hope lags behind Rae and Providence, which may indicate migration from the south. Three posts (McPherson, Aklavik, and Arctic Red River) draw from the area that was served by McPherson alone in the earlier period. McPherson and Arctic Red River are of the 10-year type of cycle while Aklavik, the most northerly post, is of the four-year arctic type of cycle. The combined data for all three posts illustrates how the population curves can be complicated by the use of heterogeneous data and indicates what may happen to gene frequency distributions from such regions.

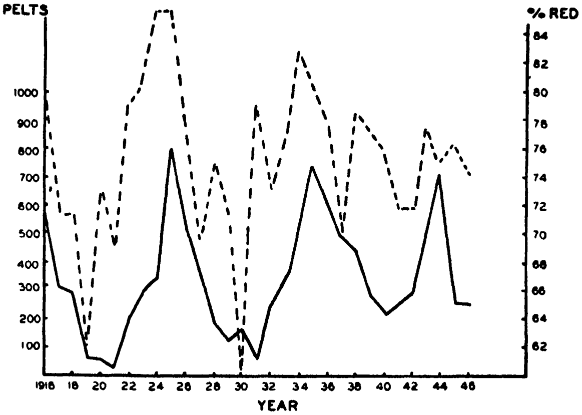

FIG. 8. *Total fox pelts taken and percentage of the red phase, Fort Rae;* ——— *total pelts,* ---- *% red.*

Many sources of error are liable to occur in data such as these. In the first place, the use of pelt numbers as a criterion of population changes presupposes that the proportion trapped each year bears a definite relationship to the total available population. We have no statistical evidence on this point and some observations indicate that animals are easier to trap when they are plentiful and often trap-shy and wary when they are scarce; the percentage trapped in a peak population will be greater than in a low population. If this is so the pelt figures will still indicate the population ups and downs but will distort the magnitude. The effect of this possible error on the colour phase ratio should be negligible unless one colour phase is more easily trapped than the others. Another cause of error in the 1860 series is that the fur from some outposts did not always reach the parent post in the year that it was trapped and consequently it was included with the following year's fur.

These and other possible sources of error must be considered since we have no way of eliminating them from the 1860 data.

General Distribution of the Colour Phases

Although there are yearly fluctuations in the proportions of the different colour phases, a general picture of the regional distribution can be obtained at each post for the two periods. When this is done we get the following series, which are illustrated in Figs. 1 and 2:

	Silver		Cross	
	1860–93	1915–45	1860–93	1915–45
Fort McPherson	12.0	5.23	42.7	37.7
Fort Good Hope	8.6	4.10	37.4	33.3
Fort Norman	8.8	3.97	36.3	32.8
Fort Providence	9.7	2.03	35.8	21.0
Fort Rae	8.5	2.56	33.7	20.0

Examination of these figures in relation to the geography of the post (Fig. 1) shows cross fox in a perfect descending series from north to south in both periods. The percentage silver shows a similar descending series but it is not as regular as that for cross fox. Northern foxes have a much greater proportion of both silvers and crosses.

The other important point is the large decreases in the percentages of both silver and cross foxes at all posts when the 1860 series is compared with the 1915 one. There is a 6.0% (9.5 to 3.5) decrease in silvers and a 8.0% (37 to 29) decrease in cross. Although the north/south gradient is maintained the amount of decrease between the two series for each post is not uniform. Good Hope and Norman show the least decrease in both silvers and crosses. McPherson shows a large decrease in silvers and a small one in cross fox. Rae and Providence show large decreases in silver and very large decreases in cross. We can generalize by saying that in the north silvers have been decreasing relatively faster than crosses, while in the south the opposite situation prevails. Since these changes are due to a variety of causes no regression figures are given; each section will be discussed separately.

A curious difference between the 1860 and 1915 series is that when the percentage of red is plotted against the percentage of cross for each post, the co-ordinates of the 1860 series all lie below the curve of the theoretical value on a monofactorial basis, and the co-ordinates of the 1915 series all lie above this curve. Thus on a single factor basis the cross fox in the 1860 data are approximately 2% short of the expected whilst in the 1915 data they are 2% more than expected. To compensate for this the silver is higher than expected in the old series and lower than expected in the 1915 series. A possible explanation for the higher percentage of silvers in the early series lies in their greater value at that time. Trappers forced to travel great distances through almost inaccessible country to market their furs would carry one silver pelt rather than the 10 or more reds required for equal value. Also a few furs are utilized by the natives for parka trimmings, etc., and these would tend to be the cheaper reds rather than the silvers.

In the north/south series of five posts previously discussed, Fort Simpson, lying between Norman and Providence, was omitted on purpose. This post lies at the junction of the Liard and Mackenzie Rivers and seems to be more influenced by the drift down the Liard than it is by the Mackenzie. It appears to fit into the following series:

	Silver		Cross	
	1860–93	1915–45	1860–93	1915-45
Fort Simpson	12 0	2 93	39 6	28 9
Fort Liard	16.2	4 10	42 5	30 2
Fort Nelson	17.0	4.05	44 1	33 0

The percentage of both silver and cross increases as you move up the Liard River and into northern British Columbia. Both the 1860 and the 1915 series show this trend very well although there has been a very large reduction in both the percentage silver and the percentage cross between the two series.

Population Genetics

We have already pointed out a number of differences between the colour phase ratios at the various posts. In view of these differences it seems advisable to take each region separately in considering the distribution of coat colour genes.

Fort McPherson is situated in the delta of the Mackenzie and is the most northerly collecting centre in the 1860 series. The distribution of colour phases collected by this post in the period 1860-93 was 12.0% silver, 42.7% cross, and 45.3% red. Fitting this to a single factor scheme we get a *P* of .03, which indicates that the chances of a random deviation of this magnitude occurring are 3 in 100 samples of this size. We have too many silver and too few crosses to satisfy the single factor scheme with these lumped data. The two factor hypothesis used in the last paper does not fit the data any better, and in view of later evidence it seems unlikely that the Canadian gene *b* was in this area at such an early date. Failure to obtain a fit by either method suggests that there may be large yearly fluctuations in population. It would be better to work on a yearly basis, but numbers are so small during population lows that some grouping of data seems desirable. To get a fairly homogeneous grouping of the yearly data the percentage of red was plotted. Examination of this graph showed the first 10 years to be very erratic, the next 16 years showed chance fluctuations about a line of decreasing percentage of reds, and the last seven years showed a sharp increase in the percentage of reds. Concomitant with this last increase in reds there was a decrease in the average yearly number of pelts from 334 to 95.

Dividing the series up in this manner and again testing for single factor inheritance we get the following χ^2 values:

1860–70.......	33.7
1871–86.......	.567
1887–93.......	.37

It appears that all ratios except the first 10 years are explainable by the single factor hypothesis. Testing the data for heterogeneity we find that the only group that is significantly heterogeneous is the first one. The χ^2 for heterogeneity for the 1860–70 values is 36.42 with nine degrees of freedom, a value that is highly significant. Looking over the first 10 years, we find that two years with the following ratios are responsible for the high χ^2 value:

1861.......	7.5% silver	24% cross	68.5% red	280 pelts
1868	18.2% silver	36.9% cross	45.9% red	375 pelts

If these are omitted from the data the χ^2 falls to 5.56, a value that is quite probable in such fluctuating data. At the same time the heterogeneity value falls to 6.66, which is not significant. Thus all years with the exception of 1861 and 1868 can be explained by the single factor hypothesis.

A full explanation of these two discrepancies can be given only when and if historical material becomes available for this section. Both of these years were peak years for white fox so two theories can be advanced to explain these exceptions. First, in each of these years the fur was not drawn from the whole area normally covered by the post; the presence of large numbers of white fox induced trappers to go to coastal and barren land regions instead of inland. Since the topography of this area is conducive to the formation of pockets with different gene frequencies, then the omission of some pockets with different gene frequencies would distort the ratio. That there are such pockets in this area will be shown when we analyze the recent data. Second, migration into this area of southern type foxes may have distorted the ratio.

Forts Good Hope and Norman have similar phenotypic ratios and may be considered together. Testing total data for single factor inheritance we get a very poor fit. If we split the data up into groups of more constant ratios we get the following χ^2's:

1861–70.............	38.9 (omitting 1861 and 1869) 6.6
1871–80.............	0.30
1881–93........... ..	4.94

These results are similar to those at Fort McPherson in that with the exception of two years all ratios can be explained by the single factor inheritance. It is interesting to note that one exceptional year is the same in both cases, while the other exception consists of two following years 1868 and 1869.

Fort Providence is situated at the point where the waters of Great Slave Lake pour into the Mackenzie River. It should be little influenced by foxes from the barren lands but would be one of the first posts reached by a migration from the good fox country to the south. Testing the total 1860 data for single factor inheritance we get a χ^2 of 22.9. Plotting the data we find some very erratic changes in the ratio that may account in part for the large χ^2. After dividing them into fairly homogeneous groups we get the percentages silver and cross, the yearly mean number of pelts, and the χ^2 given in Table I.

TABLE I

COLOUR PHASE DISTRIBUTION OF PELTS TAKEN AT FT. PROVIDENCE

	Silver, %	Cross, %	Mean no. pelts	χ^2
1862–69	14 4	34 6	34	21.6
1862–69 (omitting 1863, 1864, and 1867)	9.2	36 3	31	1.71
1863, 64, and 67	23 0	26.5	38	66.4
1870–78	12.1	40.2	38	4.3
1879–81	8.7	26.2	27	13.5
1882–84	7.6	47.3	66	3.2
1885–93	6.6	32.2	63	6.1

From this table we find that there are six years that are not compatible with the single factor scheme. The first grouping of 1862–69 gave a large χ^2 of 21.6 and a very large heterogeneity χ^2 of 43.35 with 7 degrees of freedom. The three years 1863, 1864, and 1867 account for most of the discrepancy and when they are subtracted the chi square drops to 1.71 with no significant heterogeneity.

The years 1879–81 do not fit the single factor ratio and form a homogeneous group. Both groups are characterized by low percentages of cross fox and the first group has an exceptionally high percentage of silvers. No explanation for these exceptional years will be advanced until we have examined the collections from the surrounding posts.

The relation between the percentage cross and the total foxes at this post is a varied one. In the 1864 population low the percentage cross is at its lowest point. At the 1872 low the percentage cross reaches one of its highest points and is consistently high all through the seven years of the population low. In the 1880 low the crosses again become very scarce, but in the last two years of this low the crosses become more numerous and reach their highest percentage one yeat before the population peak. There seems to be no correlation either positive or negative between population peaks and the percentage cross in this case.

Fort Resolution lies on the south shore of Great Slave Lake. Unfortunately the early data only go from 1862 to 1877. When the data are examined it is found that two years, 1866 and 1870, have exceptionally low percentages of cross fox and high percentages of silver. If we omit these two years and then divide the data into four groups we get the following results:

	Silver	Cross	Mean	χ^2
1862–71 (minus 1866 and 1870)	7.7	34.3	66	5.3
1866 and 1870	14.8	23.2	91	38.4
1872, 1876, 1877	7.9	38.7	136	0.3
1873–75	13.0	29.4	77	28.7

From these figures it would appear that the first 10 years, with the exception of 1866 and 1870, could be explained by the single factor inheritance. The second period of six years with a chi square of 13.0 is not explainable by single factor inheritance. It has too many silvers. Splitting it into two groups of three years each we find that the years of low silver percentage are explainable by the single factor theory, whilst the other three years (1873, 1874, and 1875) have too many silvers and too few crosses to fit into this scheme.

Fort Rae on the north arm of Great Slave Lake is greatly influenced by conditions in the barren lands. The ratios fluctuate a great deal and it is necessary to make groups of only two or three years in order to keep the data homogeneous. The tests for fit to a single factor scheme are given in Table II.

TABLE II

COLOUR PHASE DISTRIBUTION AND YEARLY PELT NUMBERS AT FORT RAE

	Silver, %	Cross, %	Mean no. pelts	χ^2
1862–68	7.5	36.3	207	3.4
1869–70	11.1	34.2	152	12.7
1871–75	7.4	30.0	256	40.7
1876–78	9.8	39.3	154	1.8
1879–80	12.7	29.2	106	17.5
1881–83	8.0	35.4	71	1.3
1884–85	13.1	29.7	133	39.2
1886–87	7.5	27.5	140	12.6
1888–90	6 2	35.7	75	0.3
1891–93	13.7	38.0	32	3.4

From this table we find that there are 19 years whose ratios could be explained by a single gene and 13 whose ratios cannot be so explained. The years that are not fitted by the single factor hypothesis lie in two main groups, 1869–1875 and 1884–1887. Each of these periods is started by two years of very high percentage of silver. We find that these nonconforming years with a high percentage of silver can be grouped as follows:

1869 and 1870	11.3% silver
1879 and 1880	13.3% "
1884 and 1885	13.3% "

It is interesting to note that the years of abundance in the white fox population were 1869, 1878, and 1884. The three high silver exceptions came when foxes had peaked and were migrating south from the barren lands. Thus it appears that the extra admixture of northern blood may be responsible for the distortion of the coat colour ratios.

Inspection of the table shows also that years when less than 100 pelts were taken fit the single factor theory. In such years it is probable that all foxes taken are local stock and that the indigenous population at this time contained only one main coat colour gene. At the times of high populations outside this area we get a migration into the area of either northern type fox or southern type fox. Each of these types have a different ratio and either may possess another coat colour gene at a different locus or of a multiple allelomorphic series.

In view of the small population numbers involved and the lack of historical information about the collecting activities of the posts at this time, it does not seem advisable to formulate any elaborate theory to explain these discrepancies Inbreeding with its concomitant increase in homozygosity could account for two of the exceptions but not the others, which show a significant increase in silvers with no accompanying decrease in cross fox. Random fluctuations in such a small partially isolated population undoubtedly account for many of the fluctuations but there are enough residuals to lead us to suggest an alternate explanation. If we regard these figures as a true sample of the population (a point open to dispute in these older data) then we must postulate selection of a fluctuating type or migrations from an area of different gene frequency with the migrants probably carrying the Canadian mutant silver gene.

Turning next to the Fort Simpson, Liard, and Nelson series, we find that the yearly numbers are very small. Also, the inaccessibility of the country makes the discarding or local utilization of inexpensive pelts more likely. At Fort Simpson there are always a few more silver pelts than would be expected under the single factor hypothesis, yet no particular year or pentad accounts for this difference. The χ^2 tests by pentads are consistent with a single factor hypothesis, yet the χ^2 (15 48) for the total indicates a very bad fit. The test of heterogeneity reveals that the data are homogeneous, so the disparity in the total ratio seems to be due to the accumulation of all these small excesses of silver. Forts Liard and Nelson show the same condition of a slight cumulative excess of silvers.

The population data reveals neither sharp fluctuations nor cycles. The trapping pressure was probably very low since there were great numbers of more valuable and less bulky furs available in this region at this time. Since populations of fox in this region are more isolated and exist in smaller numbers than in the rest of the range, the possibility of inbreeding with increasing homozygosity was investigated. The homozygosity varied from .64 to .67 but showed no evidence of a distinct trend so migration must be enough to counteract the isolation.

Genetics of the 1915-45 Series

Before attempting to analyze the recent figures it is necessary to know a little more of the history of Fort McPherson. In the period 1860–85, Fort McPherson with its outpost, Pierres' House, was collecting from a large area comprising the Mackenzie Delta, the Porcupine watershed, and North coast of Yukon and the Arctic coast to Baillie Island. Before the recent data start, Fort McPherson area had been divided up among the following posts: Arctic Red River, Aklavik, Hershel Island, and Kittigazuit. During the 1915–45 period, Hershel Island and Kittigazuit were closed out and Tuktuk and Coppermine opened up.

Evidence that the gene frequency is not the same all through the region, at least not in recent times, is presented in Table III.

TABLE III

PELTS COLLECTED FROM 1915–44

——	Silver	Cross	Red	Total pelts	Yearly average	χ^2
Fort McPherson	.0523	.377	.571	2483	83	2.6
Aklavik	.0302	.326	.642	9569	330	69.2
Arctic Red River	.060	.391	.549	4019	134	1.18
Kittigazuit	.072	.398	.530	501	42	0.03
Tuktuk	.0298	.380	.592	2878	262	37.47
Coppermine	.0257	.275	.700	5172	270	0.62
Hershel Island	.036	.330	.632	1303	77	0.91
Good Hope and Norman	.043	.348	.609	8472	326	3.60

The percentage silver varies from 2.5 to 7.2, cross from 27.5 to 39.8, and red from 53.0 to 70.0. From the χ^2's in the last column of Table III we see that all posts with the exception of Aklavik and Tuktuk fall into the single factor theory.

Aklavik and Tuktuk are adjacent posts. The yearly collections at Aklavik have been influenced by the opening and closing of surrounding posts. Hershel Island closed in 1933, Kittigazuit closed in 1928. Tuktuk did not open until 1935. The collection from 1915–30 was presumably obtained in the immediate vicinity owing to the operation of these other posts. The average yearly collection for this period was 116 compared to 455 for the next 15 years. Testing the first 15 years we get a χ^2 of 4.68 so this earlier period is compatible with the single factor interpretation. In the period 1930–1945, the first 10 years are characterized by too high a percentage of cross and too low a percentage of silver.

The only years that fit a single factor hypothesis are 1931, 1935, 1936, and 1937. The χ^2 for each of the other years is much too high. In the period 1942–1946 the χ^2's are compatible with a single factor interpretation but the heterogeneity factor is significantly high. There is a definite trend in both the percentage cross and red, the lines of best fit being:

Cross................ $Y = 20.8 + 1.058\,X$
Red................. $Y = 69.77 - .620\,X$

In the years 1922–42 the crosses were increasing at the rate of slightly over 1% per year. The past five years have not been on this line and it is too soon to say whether this represents a change in trend or merely a chance fluctuation from the previous trend. The percentage of red shows a decrease of 0.62% per year in the period 1922–42. This decrease in reds is noteworthy since it is opposite to the general trend for the region. When the fluctuations of the reds are plotted about the trend line they tend to show a cyclic tendency with 4, 6, 5, and 4 years between consecutive peaks. The crosses show a less regular cyclic tendency.

At Tuktuk, which opened in 1935, all years except 1937 and 1942 fit the single factor hypothesis when tested separately but there is too much heterogeneity in the 12-year composite figure to get even a remote fit. No trend is evidenced in these data but there is a cycle in the reds with peaks in 1935, 1939, and 1944. The significant point about these peak percentages of reds is that they all occur at population minima.

At McPherson and Arctic Red River the trend in red fox percentages is less than at Aklavik and in the opposite direction the equations being:

McPherson........... $Y = 55.6 + .130\,X$
Arctic Red River...... $Y = 44.0 + .571\,X$

It will be noted that these trends that have their origin at 1915 will come to the same percentage reds in 28 years. The red data for the two posts for the past seven years are identical. The percentage cross also shows a negative instead of a positive slope as in the Aklavik data.

The equations for the cross fox are:

McPherson.......... $Y = 38.24 - .064\,X$
Arctic Red River..... $Y = 46.50 - .391\,X$

The slope in the McPherson data is very small and reveals little change in either the cross or the reds.

Examining the recent series for Fort Good Hope and Norman, we find there is a distinct break in 1930. Prior to that time the silvers averaged 6.0% and after that date they dropped to 2.5%. There was no change in the cross percentages. Grouping the data for each of the two periods we get a χ^2 of 0.08 for 1915–30 and a χ^2 of 46.0 for 1931–46. There is evidently some discrepancy in the data of the second period, which causes us to use other methods of analysis. The difference between the sum of the χ^2's and the χ^2 of the sum is not significant, therefore the data are not heterogeneous. Furthermore, the χ^2 for the individual years are very low and indicate no significant departure from the single factor hypothesis except in 1930 and 1934. The reason for the lack of fit in the 1930–46 data is the shortage in the percentage of silver. This scarcity of silvers is not enough in any one year to cause a significant departure from the expected, but the accumulation of these shortages causes a very significant departure in the grouped data.

Examining the recent series for Forts Good Hope and Norman, we find that while the pelt figures show a well-developed 10-year cycle that fluctuated from 100 pelts per year in lows to 600 pelts per year in highs, there is no upward trend. The last low, however, is twice the magnitude of the preceding ones.

The colour ratios in toto and by years are compatible with a single factor interpretation. There is a trend in the percentage of reds that gives an average increase of 0.35% per year. The interesting point about these percentages is that they exhibit a four-year cycle that is independent of the 10-year population cycle. Further study of the highs in percentage of reds shows that they correspond roughly with the arctic four-year cycle. The estimation of the number of migrants that may have come into the section and influenced the ratios is complicated by the fact that such migrants fall into two classes; those that come into the region in the fall and are trapped the succeeding winter before they have a chance to influence the genetic composition of the indigenous stock, and those that become established in the breeding population. These latter migrants do not affect the ratio until the year after immigration when their gene frequency is superimposed on the population and leads it to seek a new equilibrium.

In Fig. 5 is plotted the pelt numbers and percentage red phase for the fox taken at these two posts. The trend in reds and the population cycle are quite obvious. In order to get some indication of the possible number of migrants the population figures have been smoothed to the 10-year cycle. The shaded area lying between the frequency polygon of pelts actually taken and the smoothed 10-year cycle is taken to give a rough indication of the number of migrants. Supporting evidence that migrants may come into the area is found in the secondary cycles the peaks of which show up in 1921, 1931, 1940, etc.

Assuming that these proportions of migrants are reasonable estimates of the number of migrant pelts in the collection, we can use these values for the basis for further calculations. For instance, in 1931 there were 60 native fox and 96 migrants, and, in 1932, 51 natives and 50 migrants. These migrants must have come from the north (the only area with excess population in that vicinity at that time) and would have colour ratios typical of the northern stock. The ratio for the indigenous population was 6% silver, 34% cross, and 60% red, which is fairly close to the expected for a single factor at equilibrium. The ratio for northern stocks was 3% silver, 38% cross, and 59% red, which is not a single factor equilibrium ratio. The actual numbers and expected numbers if all pelts came from the indigenous stock would be:

	Silver	Cross	Red
Actual...........	9	96	152
Expected........	15	87	155

$$\chi^2 = 3.34$$

This is close enough to expectation that no other explanation need be sought but the constant bias of too few silvers and too many cross makes another explanation desirable especially since the χ^2 of the total gives a significant difference. Assuming that the proportion of natives and migrants is as stated stated above, then using the appropriate ratios we get the following:

	Silver	Cross	Red
Actual..........	9	96	152
Expected........	11	93	153

The fit obtained by this method is much closer than that obtained in the absence of migrants. However, since two assumptions must be made (i.e. number and colour ratio of migrants) before calculations can be performed, the extensive use of this method is problematical. A further complication is that this method makes no allowance for the migrant individuals that stay and breed thus adding their offspring to the colour phases of pelts found in future generations.

Confirmatory evidence for this hypothesis of migration is obtained from an examination of Fig. 5. It will be seen from this graph that each peak in the percentage of reds corresponds to a shaded area of the population cycle. In other words, a probable migration into the region causes upsurge in the percentage reds. This statement is important as it provides evidence against the selective force of mere population numbers developed and analyzed thoroughly by Calhoun (3).

In view of this migration hypothesis it is necessary to go back over the data already presented and re-examine it. Before doing this it is necessary to clear up one point. The $p^2 : 2pq : q^2$ formula used as the criterion of the presence of one or more coat colour genes is based on the equilibrium condition in an extensive population (Wright (9–12)). We have been using it for small partially isolated populations where it only holds when migration, mutation, and selection are of a certain magnitude. We have assumed that mutation is negligible and need not be considered. Migration in most cases has been considered just sufficient to prevent inbreeding, while the role of selection has not been considered so far.

When we analyzed the data for Aklavik, we found a very evident decreasing trend in the reds and that all but seven years were compatible with a single factor interpretation. These seven years do not fit into a two factor arrangement either, so in view of the fact that the other coat colour alleles were not present at Fort Good Hope and Norman, it seems advisable to postulate migration in this case also.

In Fig. 4 are plotted the population numbers and gene frequency of the red pelt-producing allele (assuming single factor inheritance) for Arctic Red River and its adjacent post Aklavik. The opposing trends in gene frequency are plainly seen in this graph. Prior to 1930 population numbers were small, gene frequency fluctuations violent, and the two populations far apart in their gene frequencies as can be seen by the following example.

1925

Aklavik	25% cross, 73% red.
Arctic Red River	42% cross, 47% red.

After 1930 there was a sudden upsurge of population especially at Aklavik and the gene frequencies at the two posts became almost identical. This can be clearly seen from Fig. 4. The color ratios in 1936 were identical, both being:

40% cross, 56% red.

From the trend of these gene frequencies it is plausible to suggest that the upward trend of red foxes at Arctic Red River and the downward trend at Aklavik are due to the mixing of the two sets of frequencies by migration in both directions. If this is the case, then we should expect that in some years three classes of fox would be present and equilibrium ratios would not be obtained.

Fort McPherson data are intermediate between those at Aklavik and Arctic Red River throughout the 27 years. Since there is no pronounced increase in the population at this point, it seems that there has been no increase in the movement of animals to or from this section. The Fort McPherson collecting area probably includes part of the gene frequency range of Aklavik and Arctic Red River since it is situated between them.

At Tuktuk the heterogeneity is probably due to similar migrational movements with the possible complications from the four-year arctic cycle. The high percentage of reds that occur at population minima are of the typical western arctic type. Coppermine, farther to the east, had 70% red compared with Tuktuk's 59% red (see Table III). The population at Coppermine, Bathurst Inlet, and other Western Arctic points does not seem to reach equilibrium gene frequencies. The yearly collections are so small that the chance deviations obscure any trends due to migration or selection.

At Fort Simpson, which lies at the junction of the Mackenzie and Liard Rivers, we find that the percentage of red and cross fluctuates with the population. Reds show a correlation of +.32 and cross show an inverse relationship. There is a definite cycle in both the cross and the red figures but there is no upward or downward trend similar to that observed in the population figures. The only abnormality observed is that in the last peak the reds did not increase or the cross decrease as quickly as expected. Testing these data for single factor inheritance, we find that there are two periods that will not fit. These are 1924–1928 and 1938–1945. The first is a population peak with the precentage silver too high and the second occurs when the cross and red percentages reacted abnormally and the percentage silver was too low.

Both of these abnormalities can be explained best by migration. In 1924–28 migration from the adjoining Providence area would affect the ratio as follows:

		Silver	Cross	Red
700	Native 6.0S; 31C; 63R =	42	217	440
627	Migrants 1.7S; 20.3C; 78R =	11	127	490
1327	Pelts 4.0S; 26C; 70R =	53	344	930
1327	Actual pelts 4.0S; 25C; 71R =	54	335	938

Similar explanations can be worked for other nonconforming ratios. Imposed on this simple picture will be the complications arising from the migrants that stay and breed.

Forts Liard and Nelson lie southwest of the other posts and their collecting area is more or less confined by mountain ranges. The country does not provide good habitats for fox, consequently the collections are always small. The population is cyclic with a very pronounced trend, each succeeding peak being 40 to 100% higher than the preceding one. There is also a definite trend in both the cross and red percentages, the straight line relationship being:

$$\text{Cross} \ldots\ldots\ldots \quad Y = 43.4 - .484\,X$$

$$\text{Red} \ldots\ldots\ldots\ldots \quad Y = 50.3 + .553\,X$$

From these equations we see that reds are increasing at a slightly faster rate than cross are decreasing; the difference is made up by the decrease in silvers. Besides the trend line there is also a cyclic movement of reds and crosses. When the population increases the percentage red also increases and the cross decreases.

Testing for monofactorial inheritance we find no significant departure from the expected. All yearly and total pelt numbers could be explained on the basis of a single factor; the increase in population and increase in percentage red being due to immigration from areas of different gene frequency.

At Providence and Hay River, which draw their furs from the vicinity of the western end of Great Slave Lake, we find that only part of the yearly data are fitted by the single factor hypothesis. The population here is cyclic and the ratios obtained at the peaks will not fit whereas those obtained in the troughs will fit. These posts collect a lower percentage of cross than any other post so far encountered. In population highs the discrepancy in single factor ratios arises from too high a percentage of silvers in proportion to the crosses. Both red and cross percentages have cyclic fluctuations similar to the general population. For the first two cycles, the percentage of reds increases along with the population and reaches its peak just prior to the population peak (Fig. 6). From 1938–46 this relationship no longer holds. The reds seem stabilized at a higher level until 1943 and then decrease while the population is still increasing. The cross percentage shows the opposite relationship for the first two population cycles. It increases when pelt numbers decrease and reaches its peak at the population low. Since 1937, it has also been irregular and the opposite relationship seems to hold.

Fort Resolution lies on the south shore of Great Slave Lake and draws most of its collection from the south and east end of the lake. It is nearest to the good fox country of Northern Alberta and Saskatchewan and therefore most likely to be influenced by a migratory influx. The population graph (Fig. 7) shows a typical 10-year cycle with increasing peaks. The percentage of reds shows what appears to be a four- to six-year cycle about a decreasing trend of $Y = 78.5 - 0.269\,X$. The ratios prevailing during the first and second population lows and the last high are consistent with the single factor theory but the other ratios are not.

At Fort Rae, on the north arm of Great Slave Lake, we find that the population again exhibits a clear-cut cycle with no trend. The only noteworthy departure from the normal rhythm is that the collections in the last low are two to three times greater than in previous lows. In Table IV the data are grouped by years having more or less the same proportion of silvers, cross, and reds. It is apparent from the χ^2 column that only in the past three years could the ratios be explained by the single factor theory. The percentage of reds again shows a direct correlation with population changes. The magnitude of the variations in percentage of red in the earlier years is much greater than at any other posts. This can be seen quite clearly by comparing Fig. 8 with Fig. 7; the same scale is used in each.

It is apparent from the χ^2 in Table IV that the deviations from a single factor ratio are much larger than would be expected by chance, and that some other explanation will have to be sought. The one lone exception is the period 1943–1945. It is also apparent that the two lows and the two highs have very similar ratios as can be seen below:

TABLE IV

COLOUR PHASE DISTRIBUTION OF PELTS COLLECTED AT FORT RAE

	Silver, %	Cross, %	Mean	χ^2	n	q
1916–22	3.46	23.7	154	11.3	.14	.125
1923–26	2.32	14.8	488	52 6	.082	.127
1927–33	3.96	23.7	212	30.0	.14	.150
1934–36	2.24	17.7	610	40.1	.095	.148
1937–42	2.77	21.3	325	16.1	.124	.105
1943–45	1.48	22.3	495	0.2	0	.127

	Lows		Highs	
Silver...............	3.5	4.0	2.3	2.2
Cross...............	23.7	23.7	14.8	17.8

The irregular nature of the fluctuations over the last eight years is shown in their nonconforming ratios. The percentage of silver is the same as for the highs, and the percentage of cross is similar to the lows.

From the differences in the ratios between the highs and the lows we deduce that the composition of the population is not the same in periods of population abundance as it is in times of scarcity. When fox numbers are low, the foxes taken are apt to be of local stock and represent the typical ratio for that district.

When the population begins to increase, it is aided by migration from other sections of the country. There is evidence that the cycle in foxes does not reach its peak in all parts of the country at the same time. The first upswing in population cycle comes in the Lake Athabaska and Peace River area and spreads north, south, and east from here. The Rockies form a partial boundary to the west and the British Columbia cycle seems independent. As supporting evidence for this point, the times of the peaks of the 1934–36 cycle can be quoted. The peak year was 1934 in the Athabaska and Peace River area, 1935 in the Resolution, Rae, Providence, and Simpson areas and parts of British Columbia. By 1936 it had spread to the rest of British Columbia and the delta of the Mackenzie. Other peaks show a similar consecutive order although the lag is not always the same.

A corollary theory is that the gene or genes concerned with coat colour also influence the propagative or survival ability of their carriers. Thus, at Fort Rae, in times of fox population scarcity when interspecific strife is low and cubs are better cared for, the silver-producing gene is favored and a higher percentage of silvers are raised to trapping age. In times of abundance the interspecific strife is higher and the silvers are less favored and a greater percentage of reds is produced. If such were the case the percentage of cross would show proportionate increases and decreases in the following year.

Applying the dihybrid interpretation of Warwick and Hanson (1) and using the formula suggested by Calhoun as a simplification and extension of Butler (2) we get the series of figures in the last two columns of Table IV. The formulae suggested by Calhoun are:—

$$n = \frac{C}{C + 2R}$$

$$q = \frac{m^2 - R}{m^2}$$

C = frequency cross, R = frequency reds

$n + m = 1$ = frequencies of Alaskan gene A/a

$p + q = 1$ = frequencies of Canadian gene B/b

It will be seen from Table IV that during the first two peaks there was a regular relationship between the population highs and lows, Column 3, and the frequency n, Column 5, of gene a. While the frequency q of gene b increased steadily the frequency n has fluctuated being highest (0.14) at times of population lows and smallest (0.082 to 0.095) at times of population highs. In the 1937–43 low, the percentage silver and cross did not get as large as in past lows and so neither frequency n or q is as high. However, *n* does show a considerable increase, from 0.095 to 0.124 over the previous peak. In the final peak, 1943–45, a different situation arises. In 1943, at points to the south, red foxes had reached their highest peak in history, while from the northeast there was a southern migration after a peak the previous year at Bathurst Inlet. Partly because of this southern migration and partly from lack of food (rabbits were decreasing around Great Slave Lake in 1943), the spread of the southern population seems to have been directed eastward over the prairie park lands and northwestward down the Mackenzie. Because of these facts, the peak collection at Fort Rae did not rise as high as previous peaks whereas at more southerly points the peak was 50 to 100% higher than any previous one. Accompanying this population phenomena, we find that there are no longer two recessive genes present in the population. The ratio can be explained by one gene with a frequency of 0.127.

These changes in gene frequency are explained more easily by migration than by selection. When the population increases it is chiefly because of the emigration from the south. Such foxes are characterized by the low frequency (*n*) of gene *a*. Influxes of foxes of this type cause *n* to drop whenever the population increases. Little can be said about the magnitude of the fluctuations of *n* or the relative constancy of *q* since if the migration hypothesis holds the genes are not in equilibrium'and the values of *n* and *q* do not depict the true picture. It will be noted that in this case we have postulated two-factor inheritance whereas in other cases we have explained the whole discrepancy by migration. The other cases were tried with Calhoun's formula and either failed to solve or gave inconsistent results. In this case, the discrepancies seem too large to be accounted for by migration so both coat colour genes are considered as being present in the Rae, Resolution, and Providence area.

One of the difficulties in analyzing the results of a single post is that local peculiarities of management are reflected in the ratios. For instance, if the manager pays aggressively for reds and silvers but is not as aggressive in buying cross fox, then these will go to other buyers. Consequently, the resulting ratio will be low in its percentage of cross. We have a check on the Fort Rae data for 11 years in the N.W.T. figures published by Robinson (8). These figures, as can be seen below, show close agreement so Hudson's Bay Company figures can be accepted as indicative of the proportion of various colour phases trapped in the Fort Rae area.

Fort Providence shows the same characteristics as Fort Rae. The percentage of cross is highest (38.7, 20.3, and 24.4) in the population lows and

—	Silver		Cross	
	H.B.C.	N.W.T.	H.B.C.	N.W.T.
1934–1936	2.24	2.29	17.7	18.6
1937–1942	2.77	2.78	21.3	21.4

smallest (19.3 and 16.3) in the peak populations. Unlike Rae, the ratio can be explained by the monofactorial hypothesis. The population peaks are of a more abrupt nature than at Rae. After the initial rise the last peak failed to materialize and the characteristic dip in the percentage of cross does not occur.

At Providence and Hay River, it appears that the population peak is largely due to the influx of immigrants with a higher proportion of the red phase. The correlation between population numbers and percentage red is not close enough for this to be the whole answer. During the first population cycle the reds increased and decreased with the cycle (see Fig. 6). In later years, the correlation is either not as marked or is even reversed. Our interpretation of this is that prior to 1921 most of the indigenous foxes carried only the Alaskan gene. With the influx of foxes in the first population peak, the Canadian gene was brought in. Some of these foxes stayed to breed and although partial equilibrium was reached in population lows, each new wave of immigrants changed the gene frequencies. This would account for the lag between the increase in population and the increase in percentage red.

Examination of the data for Fort Resolution (Fig. 7) reveals that there is no correlation between the percentage of reds and the population cycle. There is a definite four-year cycle in the percentage of reds that can be explained either by immigration from the north during times of peak abundance or a fluctuating selectivity factor. It is hard to see how Calhoun's conclusion of gene *a* being negatively and gene *b* positively correlated with population size could be applied to these data. The idea of a cyclic selective force does not seem very plausible but until we acquire a greater knowledge of population cycles and their cause, we cannot disregard it entirely.

Discussion

The presence of the black or silver form of the red fox *Vulpes* sp. has been noted as occurring in the Canadian wild fox populations since the earliest historical records. Little attention was paid to fox fur at this time so it is lumped with miscellaneous furs; no authentic breakdown by phases is available.

The first records that give the relative abundance of the three phases and the section of country from which the foxes are drawn are those of Elton (6) for Labrador, and Cowan (4) for British Columbia. These records are both for the 19th century and show that stratification had occurred at that date. Although it appeared that there were concentrations of the silver producing gene in certain areas, it also revealed a definite north/south trend with the proportion of silvers increasing towards the north and decreasing towards the south. This appears to be a contradiction of Allen's or Gloger's rule of melanin pigmentation within a race. On the Atlantic and Pacific coasts the proportion of silvers is significantly higher than in the inland districts.

Since 1830 there has been a diminution of the silvers and an increase in the reds. Accompanying this there has been a rapid increase in fox numbers in recent years, especially in the centre of the range.

The percentage of each of the colour phases present varies with the locality and a definite cline is shown. Silver and cross are more abundant in the north than in the south and show a steady decrease as one moves southward. An exception to this is in the Liard valley. The silvers and cross increase as one moves southward up this valley from Fort Simpson to Fort Nelson and then the normal north/south cline is established for the rest of British Columbia. The percentages of silver and cross also vary with time, there being a large decrease in percentage of silvers and crosses between the 1860 and 1930 periods.

The 1860–1890 data for all posts except Fort Rae can with the exception of a few years be explained by a single pair of alleles. There are no major trends in population or gene frequencies at this time. The changes in gene frequency appear to be of the type that could result by chance in a partially isolated breeding population. Migration from the north balanced out that from the south and also overcame the effects of inbreeding. At Fort Rae, this does not hold; the ratios appear to be changed by frequent immigration, the migrants bringing in the other pair of coat colour alleles.

In the 1915–46 series, several different trends are visible. At Aklavik and Resolution the percentage of reds is decreasing while at most other posts it is increasing. At Simpson, Liard, and Nelson and to a lesser extent at Providence, Hay River, and Rae, the percentage of reds fluctuates directly with the population. At Resolution, Good Hope, and Norman, the percentage of reds shows a four-year cycle that is not correlated with the 10-year population cycle. Both these types of gene frequency changes can be explained by migration but they are harder to explain by selection. This is especially true in the case of the four-year cycle of percentages of reds.

The changes at Aklavik and Arctic Red River would also be hard to explain by selection since the changes are in opposite directions but they can be explained by periodic migrations in both directions from these areas of differing gene frequency. The mixing has now reached the point where the two areas have practically identical frequencies. At other points, it was shown that by splitting the population into indigenous and migrant, and then applying the appropriate gene frequencies, a plausible explanation of the prevailing ratios could be obtained.

Points north of Providence are, with the exception of a few years, compatible with the single factor interpretation. These exceptional years can be explained by migration. South and east of Providence, the two factor hypothesis seems necessary. At these latter points, there is ample evidence of migration and it is doubtful whether equilibrium ratios are reached.

A provisional working hypothesis of the evolution of coat colour in foxes may be advanced as follows. The mutation to black occurred in the American red fox sometime prior to 1600. At least two different gene mutations occurred, one in Labrador and one in British Columbia or Alaska. The fox population was small enough at this time that the recurrent mutations found themselves in a population that had become divided into small, partially isolated groups. Under these circumstances, differential population growth and migration made possible the establishment of the mutation and their distribution throughout the country. It may be significant to note that these mutations arose in poor fox habitats.

The two mutations are genetically different and now overlap over most of the foxes' range. The huge increases in population have taken place mostly in Alberta and Saskatchewan where the density of the silver producing gene is lowest, thus most migrants come from areas of low silver density. The increase in this region is due not to the adaptive ability of the local fox type but to the sudden changes in its habitat, such as settlement in Peace River and other northern areas.

In these small partially isolated groups the frequencies of the coat colour genes drift back and forth irregularly and might reach fixation because of the inbreeding that occurs in small populations. The local race, however, is subject to a small amount of cross-breeding with the rest of the species and the tendency towards fixation is balanced by immigration pressure instead of mutation and selection. North-south migrations would establish a cline since a greater proportion of migrants would stay and breed in adjacent areas than in distant areas. If local selection is stronger than migration pressure, well defined local races would arise.

No allowance has been made for an admixture of European red fox genes. We know that the English red fox, which lacks the coat colour mutation, was imported into the New England States for fox hunting and is presumed to have spread west and north. These genes may now be present in southern Ontario but certainly are not in the area under discussion.

We have assumed that the silver-producing gene is indifferent to selection but there is little critical evidence on this point. It is obvious that under domestication silvers can be raised as easily as reds. This cannot be due merely to the selection of the proper genetic background because outcrosses to wild stock do not affect the fecundity or viability of the silver segregants. No significant differences between red and silver litter sizes have been observed. Nothing is known about the differences in foraging ability, or predator relationships between the various colour phases, but it is often inferred that man, the arch-predator, is discriminatory (Elton (6), Haldane (7)).

One of the big problems in constructing an evolutionary theory that will hold for fox populations is the constant flux in population density. Short time oscillations in population numbers cause minor changes in gene frequency (Wright (11)). In cyclic lows the population is reduced to a few individuals with restricted range and a large amount of inbreeding. At such times there is a fixation of genes at the rate of $\frac{1}{4N}$ per generation where N = the new population number (Wright (12)). In population highs the breeding is random in areas of high density. With high density and concomitant overflow or migration to surrounding areas, at least three things may happen to the migrating foxes. (1). They may find the population of the adjacent area still relatively sparse and they could find vacant habitats in which to stay and breed both among themselves and with the indigenous population. (2). They may find the neighboring population also reaching its peak and any foxes that stay would be forced to take marginal habitats. This would decrease their chances of breeding and raising successful litters. (3). They might find the neighboring fox also at peak population and the migrants would be forced to pass right on through this territory. Needless to say, each of these conditions would affect the fur catch and colour ratio differently.

The colour phase ratio of the population would be affected two ways by immigration. If the migrants carried the same alleles as the indigenous population, there would be a more or less mechanical mixing of the two gene frequencies, and the percentage of cross and silver would increase or decrease together. If the migrants carried a different pair of coat colour alleles then the percentage silver and cross would not vary together. Both conditions were shown to occur in fox population figures (Butler (2)).

The whole problem of fluctuations and trend in gene frequencies is connected with the cyclic fluctuations and the increasing fox population. This can be explained by selection (Haldane (7), Calhoun (3)) but a simpler explanation is that of migration following population increase. Such migration coming from areas of low gene frequency and breaking down the barriers of partial isolation has resulted in a lowering of the gene frequency of the whole population.

The evidence for migration is mostly indirect and comes from the following sources:

1. In the fall of 1942 coloured foxes were extremely abundant at York Factory and relatively scarce at Island Lake 350 miles inland. After freeze-up the fox became exceedingly scarce at York Factory and abundant at Island Lake.
2. Foxes become abundant every four years in northern Quebec and every nine years in the south of the province. Consequently at certain times there is a heavy population pressure in the north and a light one in the south. At such times there is a 'run' of northern foxes, the northern type fur being taken in southern Quebec.
3. In 1943 notes were taken on the abundance of fox in a marshy area with few 'travel-ways'. The notes indicated a definite seasonal concentration on certain 'travel-ways' and a general exodus from the region.

Acknowledgments

I wish to express my appreciation of help given by the Fur Trade Department of the Hudson's Bay Company without whose co-operation this study could never have been completed.

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